# Neural Systems of Pain and Related Fear Investigated on the Basis of Painful Dental Stimulation

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# PREFACE

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## ENGLISH ABSTRACT

Using functional magnetic resonance imaging (fMRI), brain responses to acute pain can be observed "in real time" and its cortical representation has been extensively studied in the past years. However, most fMRI studies applied painful stimuli to the body periphery whereas the application of painful trigeminal stimuli e.g. to the face or teeth is rare. This is surprising since toothache is a very common form of pain with intense pain quality and frequent clinically relevant conditions such as dentine hypersensitivity. Furthermore, fear of dental pain, in its pathological form dental phobia, is one of the most common anxiety disorders among specific phobias. Therefore, the question arose whether tooth- and spinal induced pain and associated fear responses share common or differential cortical mechanisms with regard to the high prevalence of dental pain.

Study 1 examined the hypothesis whether tooth pain triggers brain activation patterns similar to that of spinal induced pain in healthy subjects.

Study 2 aimed at identifying specific brain responses of clinically relevant dental pain, namely dentine hypersensitivity, by applying a natural tooth pain stimulus (air).

Finally, study 3 investigated the question whether fear of dental pain triggers differential responses in the human fear network compared to fear of other bodily pain.

In summary, the results of the current thesis imply that dental pain elicits similar brain activation patterns as spinal induced pain, yet it features some peculiarities. Furthermore, dentine hypersensitivity pain seems to be encoded in specific brain regions of the cortical pain system. And finally, fear responses triggered by dental pain exhibit significant stronger activity within the neuronal fear network compared to equal pain of spinal origin indicating an enhanced susceptibility of tooth pain to fear conditioning.

## DEUTSCHER ABSTRACT

Mithilfe der funktionellen Magnetresonanztomographie (fMRT) können die Reaktionen des Gehirns auf Schmerzen „in Echtzeit“ dargestellt werden. In den letzten Jahren wurde so die kortikale Repräsentation von experimentell induziertem Akutschmerz intensiv untersucht. Häufig jedoch wurden Schmerzreize in der Körper-Peripherie appliziert, sehr selten im Gesicht oder an den Zähnen. Dies ist deshalb verwunderlich, da es sich bei Zahnschmerz um eine sehr häufige Schmerzform mit meist intensiver Qualität handelt und klinisch relevante Schmerzbilder, wie z.B. die dentale Hypersensitivität, eine hohe Prävalenz aufweisen. Des weiteren ist die Furcht vor Zahnschmerz, in der pathologischen Form die Dentalphobie, eine der häufigsten Angsterkrankungen unter den spezifischen Phobien. Von daher stellte sich die Frage, ob trigeminal und spinal vermittelter Schmerz und die damit assoziierten Furchtreaktionen ähnliche kortikale Prozesse teilen oder aufgrund der Häufigkeit von Zahnschmerz unterschiedliche neuronale Mechanismen aufweisen.

Studie 1 verfolgte demnach die Hypothese, ob bei gesunden Personen Zahnschmerz ähnliche Hirnaktivierungsmuster auslöst, wie dies bei spinal vermitteltem Schmerz der Fall ist.

In Studie 2 wurden Hirnreaktionen auf klinisch relevanten Zahnschmerz, die dentale Hypersensitivität, untersucht. Dies anhand eines natürlichen Schmerzreizes (Luft).

Studie 3 schlussendlich ging der Frage nach, ob die Furcht vor Zahnschmerz stärkere Reaktionen als die Furcht vor anderem Körperschmerz im Furchtsystem des Gehirns auslöst.

Zusammenfassend implizieren die Ergebnisse der vorliegenden Dissertation, dass Zahnschmerz im Gegensatz zu peripherem Schmerz bezüglich Hirnaktivierungsmuster vergleichbar, jedoch in einigen Aspekten eine Sonderrolle einnimmt. Des weiteren scheint klinisch relevanter Schmerz bei dentaler Hypersensitivität in spezifischen Hirnregionen des kortikalen Schmerzsystems kodiert zu werden. Und schlussendlich fallen die Furchtreaktionen im Furchtnetzwerk des Gehirns auf schmerzhafte Reize am Zahn signifikant stärker aus als die Reaktion auf anderen Körperschmerz, was auf eine erhöhte Konditionierungs-Anfälligkeit von Zahnschmerzen hinweist.

# 1. General introduction

Robert Burns in the 18th century penned 'Address to the Toothache' in which he describes the toothache as being worse than being tortured in hell:

*Whare'er that place be priests ca' Hell,*
*Whare a' the tones o' misery yell,*
*An' ranked plagues their numbers tell*
*In dreadfu' raw,*
*Thou, Toothache, surely bear'st the bell*
*Amang them a'!*

What is special about dental pain and related fear? Pain in the face and mouth region represents one of the most common and unpleasant pains in the body (Sessle, 2000). Toothache is the most common form of orofacial pain may have a significant impact on eating, sleep and daily activities (Scully, 2013). In particular dentine hypersensitivity, a very common but also enigmatic condition in the daily clinical practice seems to unify physical and psychological aspects in a characteristic manner (Dababneh et al., 1999; Orchardson and Gillam, 2006). Dentine hypersensitivity is characterized by a sharp and short pain experience induced by eating, drinking, brushing and sometimes even breathing (Dababneh et al., 1999) and is accompanied by a high probability of unpleasant live affecting side effects. Regarding fear of dental pain, the pathological form, dental phobia, is one of the most prevalent phobias and is a remarkably severe condition with protracted duration and resistance to treatment (Agras et al., 1969; Fiset et al., 1989; Oosterink et al., 2009). Moreover, dental phobia is unique as no other body part is associated with a specific phobia. Pain perceptions from the periphery of the body are mediated by two known components of nociception: the first rapid or sharp pain and the second dull pain are considered to be related to activation of A-delta- and C-type nociceptive primary afferents, respectively. This dichotomy of pain transmission also exists in teeth, but the sensation might not be clearly separated due to the short distance between the site of stimulation and the brain (Narhi et al., 1992). Nevertheless, dentinal stimulation of teeth with healthy pulps induces typically short and

sharp pain making it an ideal candidate for a target site for pain stimulation because of a lack of superimposed mechano- and thermosensations. The idea behind selecting a tooth as a target site for a purely nociceptive stimulus is not new (Chatrian et al., 1975). However, with the advent of novel neuroimaging methods such as fMRI we are now able to identify supraspinal neuronal responses to experimentally induced tooth pain and related fear and elaborate on their differential processing with regard to spinothalamic transmitted pain. Brain regions concomitantly activated by acute noxious stimuli have been collectively named as the "Pain Matrix" and include the thalamus, primary and secondary somatosensory cortices (S1 and S2), insular cortices, the anterior cingulate cortex (ACC), frontal cortices and the cerebellum (Apkarian et al., 2005; Duerden and Albanese, 2013; Moulton et al., 2010; Peyron et al., 2000). Recently, a meta-analysis investigating the brain representation of experimental dental pain (Lin et al., 2014) revealed that dental pain activates the core pain-related network (Pain Matrix) including the primary and secondary somatosensory cortices (S1 and S2), the insula, the thalamus, the cingulate cortex and frontal brain regions. However, compared to neuroimaging studies investigating pain from non-trigeminal origin, studies applying experimental tooth pain are rare (Lin et al., 2014). Therefore, the aim of the current PhD thesis was to identify specific brain responses to experimentally induced dental pain and to examine the hypothesis whether tooth pain triggers brain activation patterns similar to that of spinal induced pain in healthy subjects (Study 1). Second, we applied a novel approach to mimic clinically relevant dental pain, namely dentine hypersensitivity, by applying a natural tooth pain stimulus (air). This investigation should shed new light on common and differential cortical mechanisms between experimental and clinically relevant dental pain (Study 2). Finally, in a third study (Study 3) we aimed at investigating the question whether fear of dental pain triggers differential responses in the human fear network compared to fear of other bodily pain. While many dental phobics experience aversive and painful situations at the dentist, there are a number of individuals who have neither had nor recall any traumatic dental experience but do report being afraid of going to the dentist . Thus, a better understanding of dental fears and phobia would be achieved by first developing a better understanding of what is special about teeth and fear conditioning.

# 2.1 STUDY I:

## TAKING SIDES WITH PAIN - LATERALIZATION ASPECTS RELATED TO CEREBRAL PROCESSING OF DENTAL PAIN

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ABSTRACT

The current fMRI study investigated cortical processing of electrically induced painful tooth stimulation of both maxillary canines and central incisors in 21 healthy, right handed volunteers. A constant current, 150% above tooth specific pain-perception thresholds was applied and corresponding online ratings of perceived pain intensity were recorded with a computerized visual analog scale during fMRI measurements. Lateralization of cortical activations was investigated by a region of interest analysis. A wide cortical network distributed over several areas, typically described as the pain or nociceptive matrix, was activated on a conservative significance level. Distinct lateralization patterns of analyzed structures allow functional classification of the dental pain processing system. Namely, certain parts are activated independent of the stimulation site, and hence are interpreted to reflect cognitive emotional aspects. Other parts represent somatotopic processing and therefore reflect discriminative perceptive analysis. Of particular interest is the observed amygdala activity depending on the stimulated tooth that might indicate a role in somatotopic encoding.

Keywords: toothache; fMRI; dominance, cerebral; amygdala; cerebral cortex, lateralization

## INTRODUCTION

Brain structures consistently activated by noxious stimuli are: anterior cingulate cortex (ACC), insula, secondary somatosensory cortex (SII), lentiform nuclei, cerebellum, and thalamus. Less consistently, activation related to nociception has been reported for primary somatosensory cortex (SI), motor cortex (M1), premotor areas, and subcortical structures (Treede et al., 1999; Petrovic et al., 2000; Peyron et al., 2000; Bingel et al., 2002; Farrell et al., 2005). Functionally, these areas have been divided into a lateral and medial pain system and substantial evidence has emerged in support of this model (Albe-Fessard et al., 1985; Bushnell et al., 1999; Tracey and Mantyh, 2007), although alternative hypotheses have also been put forward (Apkarian et al., 2005; Craig, 2005; Mouraux and Iannetti, 2009).

Generally, the medial pain system composed of the insular cortex, anterior cingulate, and limbic structures is held responsible for processing emotional-affective and cognitive-behavioral pain aspects (Kulkarni et al., 2005; Wiech et al., 2006). The lateral pain system is

attributed to sensory-discriminative components of pain and includes the lateral spinothalamic tract, the ventral posterolateral nucleus of the thalamus, and SI (Kenshalo Jr. et al., 1988; Bushnell and Duncan, 1989; Bushnell et al., 1999). In line with these functional attributes, one would expect to find evidence from experimental pain studies showing contralateral activation in this lateral system in response to unilateral noxious stimuli. Surprisingly, human imaging studies cannot consistently confirm activation in the lateral system in response to unilateral noxious stimuli. SI for example is only activated in approximately 50–75% of all reports (Bushnell et al., 1999; Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005). Similarly, hard evidence is lacking for distinct contralateral hemispheric activation of other structures of the lateral pain system. One explanation may be that only few studies report on administering noxious stimuli to bilateral homologous body parts (Coghill et al., 1999, 2001; Bingel et al., 2002, 2003; Brooks et al., 2002; Youell et al., 2004; Symonds et al., 2006). The current study aimed at elucidating cortical spatial representation and hemispheric lateralization in response to dental nociception.

Ideally, lateralization aspects of pain were investigated by asynchronously applying bilateral noxious stimuli at graded distances to the body midline. This is readily realized by stimulation of multiple teeth as previously done (Ettlin et al., 2004, 2009). A possible interference by midline crossing of maxillary nerve endings is unlikely based on findings by Kemppainen et al. (2003). Jantsch et al. (2005) published the first brain fMRI investigation on tooth pain induced by electric stimulation. However, they stimulated one single tooth only as well as the ipsilateral dorsal hand. The results of their study suggest that brain processing of electrically evoked dental pain shows similarities as well dissimilarities compared to upper extremity mechanically induced pain.

Based on the model of a lateral and medial pain system, we hypothesized that within the cortical pain circuitry, certain brain areas be activated dependent on the stimulation side and others showing lateralized or bilateral hemispheric activity independent of the side of stimulus application.

## MATERIAL AND METHODS

### PARTICIPANTS

21 neurologically healthy subjects (8 female/13 male, age 20-44, all right handed (Annett 1970) with no dental pain experience during the preceding year participated in the experiment. Inclusion criteria required test teeth to be caries free, vital, and without attachment loss. Dental and periodontal pathologies were excluded by professional dental and radiographic examinations of maxillary teeth. Subjects received detailed information about the experimental procedure and provided written informed consent. The study was approved by the local ethics committee and was conducted according to the guidelines of the Declaration of Helsinki for treatment of experimental human subjects.

## EXPERIMENTAL MATERIAL

Maxillary alginate impressions were taken from the subjects' dentitions for fabrication of soft dental acrylic splints. Four pairs of stainless steel electrodes were embedded in each individual dental splint opposite the labial and palatal surface center of the target teeth, namely maxillary canines and central incisors (Fig. 1). They served as anode and cathode during electric stimulation. In order to minimize electric resistance during stimulation, a round piece of hydrogel (AMGEL Technologies, AG602-6, 8520 Lystrup, Denmark) with 3 mm diameter was placed between the tooth and anode and cathode, respectively, and was covered with a thin layer of toothpaste (Signal Microgranuli, Unilever, Zug, Switzerland).

Electrical stimulation was performed by means of the portable system Compex Motion System (Keller et al., 2002) and the experimental protocol was controlled by the Presentation software (www.neurobs.com/presentation) via parallel port using a self made interface. To avoid radiofrequency contamination of the stimulation current, specially shielded wires were used. For rating of the stimulus intensities within the MRI scanner, a computerized visual analog scale was used (COVAS; MEDOC, Haifa, Israel), with anchor points "no pain" on the left and "worst imaginable pain" on the right. This COVAS was projected onto a screen outside the scanner, and a mirror based deflection system enabled its visibility for the subjects.

Figure 1: Customized acrylic splint with carbon wires and stainless steel electrodes (fabricated for each subject). Electrodes were placed on the labial and oral face of the respective tooth.

## SENSORY TESTING PRIOR TO THE MR EXPERIMENT

One to two weeks prior to the MR experiment, sensory testing with the tooth stimulation setup was performed in order to assess individual thresholds for sensory perception (SPT), pain perception (PPT) and pain tolerance (PTT) separately for each target tooth. The three thresholds were defined as the average ascending electric stimulus intensity out of three tests at which the subject reported sensation, pain and pain tolerance, respectively. We also questioned subjects whether single stimuli were felt distinctly in one test tooth only, which was acknowledged by all participants. Sequence of tooth stimulation was randomized between individuals.

For all tooth stimuli (threshold determination and fMRI stimulation protocol) biphasic pulse forms of 1ms duration were applied on both maxillary canine and medial incisors with interstimulus intervals randomized between 7.5 to 10 seconds.

## FMRI DATA ACQUISITION AND STIMULATION PROTOCOL

Within one to two weeks after sensory testing, subjects underwent the fMRI protocol in a Philips 3-T Achieva system (Philips Medical Systems, Best, The Netherlands) at the same time of day as threshold determination was performed, since evidence indicates a diurnal association of somesthesic perception (Fillingim and Ness, 2000; Sessle, 2000; Wiesenfeld-Hallin, 2005). Subjects were placed in the scanner in a supine position and their individual

SPT and PPT were re-tested inside the scanner to exclude changes related to the experimental setting. No significant differences were observed (anova, greenhouse geisser corrected, F = 1.653, p = 0.187, $eta^2$ = 0.076. The fMRI stimulation protocol consisted of 40 constant stimuli per tooth applied in randomized order to the four teeth with an intensity 150% of the tooth specific PPT. Pain intensity ratings were used to control for differences in perceived pain intensity among tested teeth. For each tooth subjects were requested to rate the pain intensity of 10 randomly selected stimuli (25% of all stimuli applied). For those stimuli to be rated, the VAS appeared directly after stimulus delivery, and subjects were offered 5 seconds for pain intensity rating. For the remaining 75% of trials, the stimulus was followed by a fixation cross on the screen. We decided not to have every stimulus rated in order to minimize motion artifacts and other rating influences (Schoedel et al., 2008). All scans followed by a rating were therefore excluded from further fMRI analysis. The experimental run lasted approximately 23 minutes.

For the functional scans, a blood oxygen level dependent (BOLD) sensitive single-shot gradient echo planar imaging sequence was used with 33 axial slices, covering the entire cerebrum and cerebellum, using an 8 channel receive-only head coil. Parameters: echo time = 30 ms, flip angle = 75 degrees, repetition time = 2500 ms, slice thickness = 4 mm, inter-slice gap = 0 mm, field of view = 230 mm and matrix size in plane = 128 x 128, resulting in a voxel size of 1.72 x 1.72 x 4 $mm^3$. Three "dummy" scans were first acquired to reach steady state magnetization and discarded. 180 high-resolution T1 weighted axial slices (spoiled gradient echo) were acquired with TR = 20ms, flip angle = 20°, voxel size = 0.98 x 0.98 x 1.02 $mm^3$, FOV = 24 cm, matrix = 256 x 192, which were used as an underlay for individual functional maps.

### DATA ANALYSIS

Individual pain perception thresholds were analyzed with respect to differences between the laboratory and fMRI condition in a repeated measures analysis of variance (RM-ANOVA), with the factors "location" and "tooth". A separate ANOVA with mean COVAS ratings per tooth as dependent variable, "tooth" as within-subject factor and “gender” as between-subject factor was calculated to check whether within each subject pain intensity and PPT varies between the stimulated teeth.

Functional image analysis was done using the SPM5 (http://www.fil.ion.ucl.ac.uk/spm) software package running on MatLab R2007a (Mathworks, Natiek, USA). In a first step, spatial realignment and reslicing to the first image in the series as reference was performed (detected movement did not exceed 1.5 mm (translational) or 1° (rotational) compared with the reference image). For studying group effects, data were normalized to the MNI template brain (Evans et al., 1993) followed by smoothing with a Gaussian kernel of 6 mm (FWHM) and scaled to the global mean intensity. A General Linear Model (GLM) was set up and estimated. Differences between stimulation and baseline were transformed into colour-coded T-maps for each voxel and superimposed onto the MNI single-subject-T1 brain. Corrected data (FWE) (Worsley et al., 1996) with $p < 0.01$ are reported in the general cortical activation section. Regions of interest (ROI) were defined, based on images provided by the "WFU-Pickatlas" (Lancaster et al., 1997 and 2000; Maldjian et al., 2003) for areas selected from pain literature reviews (Peyron et al., 2000; Farrell et al., 2005): postcentral gyrus, thalamus, amydgala, supramarginal (BA40), preparietal (BA5) and superior parietal (BA 7) areas, subcentral area (BA 43), cerebellum (anterior and posterior lobe), the supplemental motor area (BA6), frontomedial area (BA 46) and frontopolar (BA 10) areas, hippocampus, parahippocampus, caudate, putamen, pallidum and the brainstem. Two exceptions were applied: the "insula-ROI" provided by the WFU-Pickatlas was divided into three parts (anterior, medial and posterior) according to Brooks et al. (2002), since several reports suggest a complex anatomical (Varnavas and Grand 1999) and functional (Coghill et al.,1999; Brooks et al., 2002 and 2005; Symonds et al., 2006) fragmentation within the insula. To take into account the functional complexity of the cingulate cortex, we subdivided this structures based on Vogt, 2005. The numbers of activated voxels, mean- and maximum activation were calculated within each ROI.

Data were then analyzed using SPSS for Windows (SPSS Inc. Chicago, Illinois, Release 14.0.0). A reapeated measurement ANOVA (RM ANOVA) with “hemisphere” and “side of stimulation” as within-subjects-factors was performed for the ROIs. Main effects for factor "hemisphere" as well as interaction between factors “hemisphere” and “side of stimulation” were analyzed. For RM ANOVAS, results were Greenhouse-Geisser corrected for non-sphericity if applicable.

## RESULTS

### PSYCHOPHYSICS

Mean stimulus intensities of the general study population during the scanning procedure demonstrated a significant within-subjects effect (F = 3.45, p = 0.02) ranged from 20.76 to 25.24 mA across the four teeth, whereas respective ratings ranged from 46.9 to 49.1but showed no significant differences (F = 0.48, p = 0.70). According to gender related differences, we found a trend in the interaction gender * stimulus intensities (F = 2.74, $eta^2$ = 0.13, p = 0.051) but no interaction according to the gender * rating interaction with F = 0.87, $eta^2$ = 0.04 and p = 0.46 (for detailed information please see Table 1).

Table 1: Mean stimulus intensities and related mean ratings during fMRI in the overall study population and differentiated by gender.

| Overall (n=21) | right | | left | |
|---|---|---|---|---|
| | canine | central incisor | canine | central incisor |
| Stimulus intensities [mA] | 25.2 ± 10.3 | 20.8 ± 11.3 | 24.8 ± 11.5 | 23.9 ± 13.1 |
| COVAS ratings [0-100] | 46.7 ± 18.5 | 48.0 ± 19.7 | 45.5 ± 19.0 | 46.9 ± 18.3 |
| Female (n=8) | | | | |
| Stimulus intensities [mA] | 21.9 ± 9.6 | 17.0 ± 7.5 | 18.8 ± 7.5 | 15.8 ± 5.8 |
| COVAS ratings [0-100] | 38.0 ± 13.3 | 43.5 ± 20.2 | 39.5 ± 18.8 | 41.5 ± 17.6 |
| Male (n=13) | | | | |
| Stimulus intensities [mA] | 27.3 ± 10.6 | 23.1 ± 12.8 | 28.5 ± 12.1 | 28.9 ± 13.9 |
| COVAS ratings [0-100] | 52.1 ± 19.6 | 50.8 ± 19.6 | 49.1 ± 18.8 | 50.2 ± 18.6 |

In the overall study population, post-hoc t-test on stimulus intensity revealed a significant difference between right central incisor and right canine (t = 3.82, p = 0.001) as well as between right central incisor and left canine (t = 2.83, p = 0.01). An additional one-way ANOVA exploring possible gender differences showed a significant difference between the stimulus intensities of the left central incisor (F= 6.30, p=0.2) and a trend with respect to the left canine (F=4.17, p=0.55). All other comparisons reached no significant level. All values are listed with respective standard deviations.

## HEMODYNAMIC RESPONSES ACROSS THE ENTIRE BRAIN AND WITHIN REGIONS OF INTEREST

Group activation brain maps (stimulation vs. base-line) are displayed in Fig. 2 and specified in supplemental table 1 (as we focus on the lateralization analyses, we disclaim from describing this patterns here more extensively). All ROIs investigated showed significant activation compared to baseline, namely postcentral gyrus, thalamus, preparietal (BA5) and superior parietal (BA 7) areas, supramarginal (BA40) and subcentral areas (BA 43), anterior, medial and posterior insula, amydgala, hippocampus, parahippocampus, both cerebellae (anterior and posterior lobe), caudate, putamen, pallidum, supplementary motor (BA6), frontomedial (BA 46) and frontopolar areas (BA10) the subdivisions of the cingulate gyrus (PCC, pMCC, aMCC, pACC, sACC, and the brainstem) (Table 2).

Figure 2:

Cortical areas activated by electrical tooth stimulation over all four teeth (2a) and with respect to both right teeth (RI and RC) and both left teeth (LI and LC) respectively (2b and 2c). Activity is projected onto the single-subject-MNI-template. Indicators at the rendered brains stand for the views: R=from right, L=from left, S=from superior, A=from anterior, P=from posterior, all brain figures are in neurological orientation. Slices from left to right: midsagital (M), coronal (C) at Y= -36 and horizontal (H) at Z= 54. Data are corrected for multiple comparison (FWE) p = 0.01 with an extended threshold of 10 voxel.

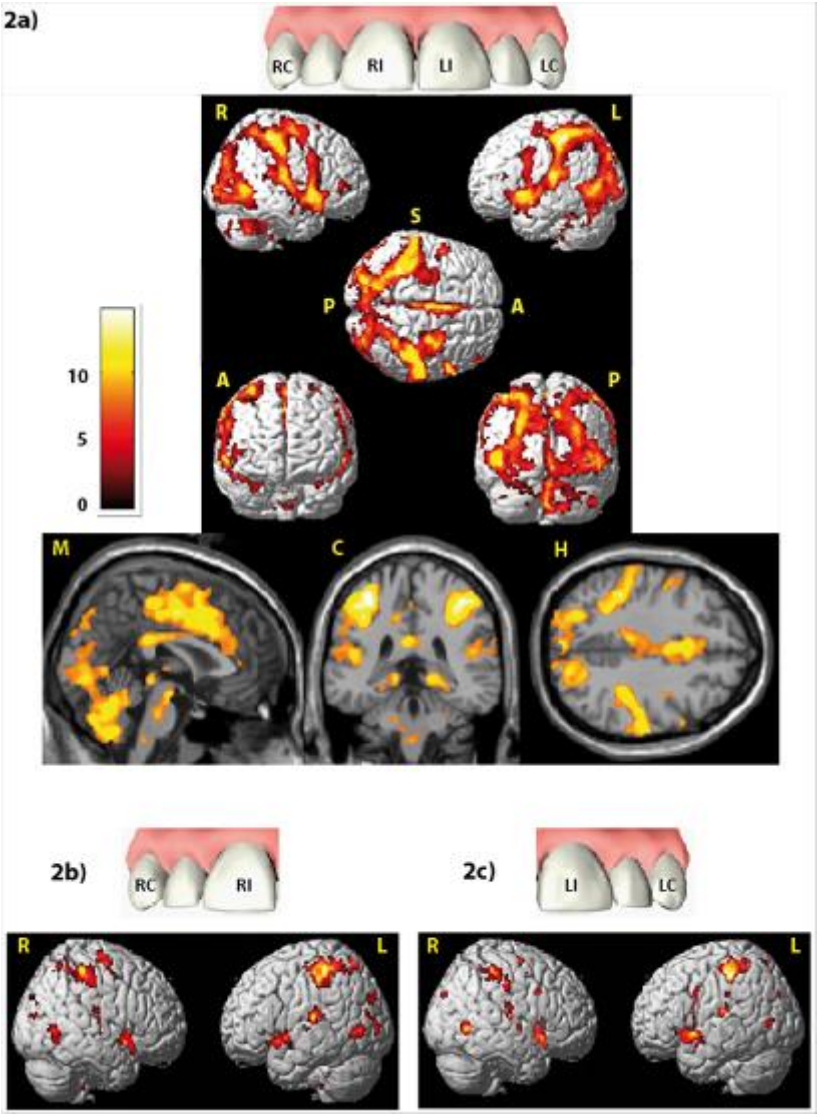

Table 2: Activation statistics in the selected regions of interest (see methods section) based on group analysis pooled across all four teeth. A small volume correction has been performed with the regions of interests as search volume. Described are cluster size, MNI coordinates of the maximally activated voxel with the respective p- and t-values. Data are family wise error (FWE) corrected ($p < 0.01$, extent voxel threshold $k = 10$).

| Anatomical Description | Hemisphere | Cluster Size | MNI Coordinates (max T Voxel) | Voxel p (FWE-cor) | Voxel T (max T) |
|---|---|---|---|---|---|
| Postcentral Gyrus (SI) | L | 970 | -38 -36 54 | 0.000 | 14.74 |
| | R | 1024 | 50 -30 52 | 0.000 | 13.16 |
| Thalamus | L | 281 | -10 -20 8 | 0.000 | 11.04 |
| | R | 275 | 14 -16 10 | 0.000 | 9.94 |
| Preparietal Area (BA 5) | L | 46 | -34 -44 62 | 0.000 | 11.13 |
| | R | 11 | 32 -48 62 | 0.000 | 7.16 |
| Superior Parietal Area (BA 7) | L | 502 | -22 -66 62 | 0.000 | 10.78 |
| | R | 387 | 16 -78 34 | 0.000 | 9.60 |
| Supramarginal Area (BA 40) | L | 586 | -40 -36 58 | 0.000 | 13.66 |
| | R | 374 | 50 -30 50 | 0.000 | 12.16 |
| Subcentral Area//SII (BA 43) | L | 9 | -52 -18 16 | 0.000 | 6.69 |
| | R | 36 | 66 -16 20 | 0.000 | 10.07 |
| Anterior Insula | L | 213 | -46 12 -8 | 0.000 | 11.38 |
| | R | 215 | 42 16 -8 | 0.000 | 10.60 |
| Medial Insula | L | 490 | -40 0 -10 | 0.000 | 11.13 |
| | R | 318 | 42 0 -10 | 0.000 | 9.85 |
| Posterior Insula | L | 52 | -44 -14 2 | 0.000 | 7.93 |
| | R | 41 | 42 -12 -8 | 0.000 | 7.92 |
| Amygdala | L | 90 | -20 0 -12 | 0.000 | 7.54 |
| | R | 72 | 26 2 -20 | 0.000 | 8.37 |
| Hippocampus | L | 79 | -20 -24 -10 | 0.000 | 10.57 |
| | R | 48 | 18 -36 0 | 0.000 | 8.62 |

| | | | | | |
|---|---|---|---|---|---|
| Parahippocampus | L | 47 | -24 -26 -16 | 0.000 | 7.80 |
| | R | 144 | 16 -38 -6 | 0.000 | 8.88 |
| Cerebellum Anterior Lobe | L | 617 | -34 -58 -34 | 0.000 | 9.21 |
| | R | 1017 | 2 -62 -26 | 0.000 | 9.96 |
| Cerebellum Posterior Lobe | L | 617 | -2 -72 -38 | 0.000 | 9.54 |
| | R | 1313 | 2 -64 -28 | 0.000 | 10.01 |
| Caudate | L | 117 | -14 16 -8 | 0.000 | 10.36 |
| | R | 108 | 16 16 -10 | 0.000 | 8.88 |
| Pallidum | L | 69 | -10 4 2 | 0.000 | 9.27 |
| | R | 6 | 16 10 -2 | 0.000 | 6.65 |
| Putamen | L | 455 | -18 14 -2 | 0.000 | 14.55 |
| | R | 264 | 22 14 0 | 0.000 | 10.23 |
| Supp_Motor_Area (BA 6) | L | 631 | -2 6 48 | 0.000 | 11.68 |
| | R | 421 | 2 8 46 | 0.000 | 10.71 |
| Frontomedial Area (BA 46) | L | no suprathreshold cluster with this conservative statistic level | | | |
| | R | 7 | 52 42 6 | 0.000 | 7.05 |
| Frontopolar Area (BA 10) | L | no suprathreshold cluster with this conservative statistic level | | | |
| | R | 1 | 52 42 0 | 0.000 | 6.41 |
| PCC | L | 280 | -8 -28 44 | 0.000 | 10.70 |
| | R | 108 | 2 -28 52 | 0.000 | 9.02 |
| pMCC | L | 249 | -2 -6 48 | 0.000 | 11.33 |
| | R | 259 | 8 -8 46 | 0.000 | 11.93 |
| aMCC | L | 521 | -2 6 40 | 0.000 | 10.37 |
| | R | 480 | 2 16 38 | 0.000 | 9.83 |
| pACC | L | 103 | -2 32 18 | 0.000 | 6.90 |
| | R | 20 | 2 34 20 | 0.000 | 6.75 |

| | | | | | |
|---|---|---|---|---|---|
| sACC | L | no suprathreshold cluster with this conservative statistic level | | | |
| | R | no suprathreshold cluster with this conservative statistic level | | | |
| Brainstem | L | 47 | -2 -34 -50 | 0.000 | 6.83 |
| | R | 69 | 2 -26 -30 | 0.000 | 7.82 |

LATERALIZATION EFFECTS BASED ON REGIONS OF INTEREST ANALYSIS

1) There are no ROIs demonstrating a significant effect for “side of stimulation”.

2) There were several ROIs that were activated strongly on one hemisphere irrespective of the side of stimulation. Both, anterior and posterior cerebellar lobes demonstrated a stronger right hemispheric effect. A stronger left hemispheric effect was found in putamen, pregenual anterior cingulate cortex (pACC), supramarginal area (BA40) and parahippocampus (Table 3, Fig. 3).

3) There was one region, namely the subcentral area (BA 43), in which “hemisphere” showed a stronger right sided effect as well as an interaction with the factor “side of stimulation” (Table 3, Fig 3). This laterality effect was observed especially after left sided stimulation.

4) There were several regions in which no main effect but an interaction between “hemisphere” and “side of stimulation” was observed (Table 3, Fig 3). Postcentral gyrus (SI), posterior insula, thalamus and amygdala all showed a hemispheric dominance contralateral to the stimulation side.

Table 3: Repeated measures ANOVA results of the region of interest analysis. Only the significant and ($p<0.05$), trend-like interactions ($p < 0.10$) are shown (see Fig.3 for illustration). Main effect "tooth" is not shown, as there is neither a significant nor a trend within that factor. F = F-Value, p = p-value, $eta^2$ = proportion of the variability in the dependent measure that is attributable to a factor.

| Anatomical Description | Main effect "hemisphere" F ( $eta^2$ ) p | Interaction effect "tooth * hemisphere" F ( $eta^2$ ) p |
|---|---|---|
| Thalamus | 0.028 ( 0.001 ) 0.870 | 11.038 ( 0.356 ) **0.003** |
| Postcentral_Gyrus (SI) | 0.876 ( 0.042 ) 0.360 | 12.928 ( 0.393 ) **0.002** |
| Posterior Insula | 0.003 ( 0.000 ) 0.959 | 4.564 ( 0.186 ) **0.045** |
| Amygdala | 3.615 ( 0.153 ) 0.072 | 23.163 ( 0.537 ) **0.000** |
| Subcentral Area (BA 43) | 17.723 ( 0.470 ) **0.000** | 12.899 ( 0.392 ) **0.002** |
| Preparietal Area (BA5) | 1.219 ( 0.057 ) 0.283 | 3.008 ( 0.131 ) *0.098* |
| Cerebellum (posterior lobe) | 18.814 ( 0.485 ) **0.000** | 1.349 ( 0.063 ) 0.259 |
| Cerebellum (anterior lobe) | 4.546 ( 0.185 ) **0.046** | 1.942 ( 0.089 ) 0.179 |
| Parahippocampus | 6.628 ( 0.249 ) **0.018** | 1.417 ( 0.066 ) 0.248 |
| Supramarginal Area (BA 40) | 7.191 ( 0.264 ) **0.014** | 1.654 ( 0.076 ) 0.213 |
| Pregenual Anterior Cingulate (pACC) | 13.934 ( 0.411 ) **0.000** | 0.771 ( 0.037 ) 0.515 |
| Anterior medial Cingulate (aMCC) | 4.271 ( 0.176 ) *0.052* | 0.507 ( 0.025 ) 0.679 |
| Putamen | 7.213 ( 0.265 ) **0.014** | 0.718 ( 0.035 ) 0.407 |
| Supplementary Motor Area (BA 6) | 3.909 ( 0.163 ) *0.062* | 0.357 ( 0.018 ) 0.557 |

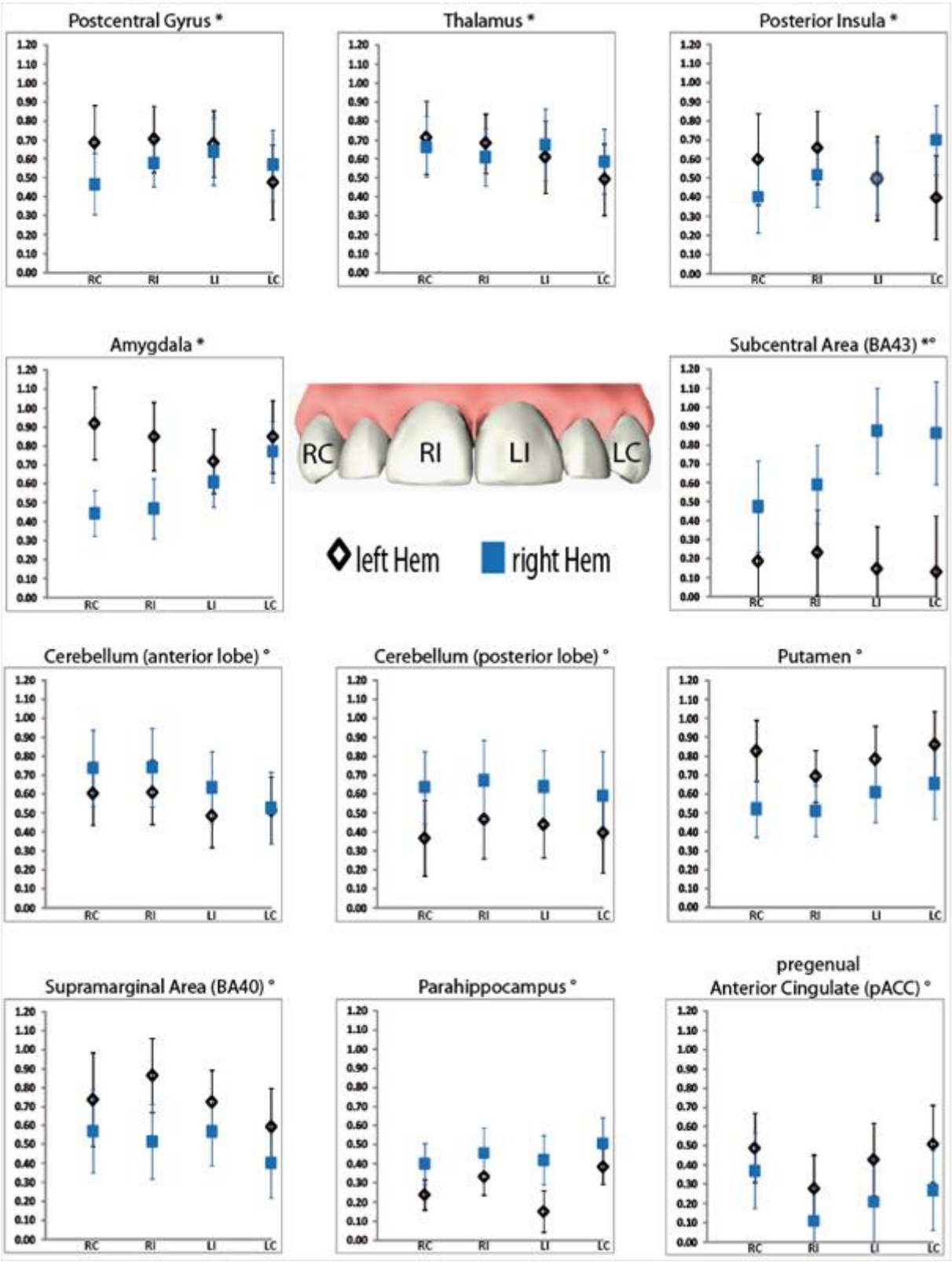


Figure 3:

Regions of interest (ROIs) showing significant main effect hemisphere (indexed with °) or interaction (indexed with *) in the repeated measure ANOVA. Displayed are mean activations (Y axis) with corresponded standard errors for each tooth within the respective hemisphere. RC = right canine, RI = right central incisor, LI = left central incisor, LC = left canine.

## DISCUSSION

The aim of this study was to elucidate cortical spatial representation and hemispheric lateralization in response to noxious electric dental stimulation. Findings reveal robust brain activation in areas previously shown to be involved in pain processing.

Focusing on lateralization aspects, we categorize the findings into three groups 1) structures exhibiting hemispheric lateralization irrespective of side of stimulation 2) structures showing activation dominance contralateral to the side of stimulation without hemispheric lateralization 3) structures demonstrating not only hemispheric lateralization, but also dependency on side of stimulation. In the following, we discuss these findings in detail.

### HEMISPHERIC LATERALIZATION IRRESPECTIVE OF SIDE OF STIMULATION

We found evidence for hemispheric lateralization in six brain areas irrespective of side of stimulation. The anterior and posterior cerebellar lobes as well as the parahippocampus demonstrate a stronger right hemispheric effect, whereas a stronger left hemispheric effect was observed in putamen, pregenual cingulate cortex, and supramarginal area (BA 40).

Pain related cerebellar activity has been consistently demonstrated (reviewed in Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005) and several suggestions are published in order to explain this often robust activity (see e.g., Saab and Willis, 2003). Evidence for direct and/or collateral trigeminal input to cerebellar structures is provided by animal studies (Snyder et al., 1978; Dietrichs and Walberg, 1987; Patrick and Robinson, 1987; Saab et al., 2001; Bukowska et al., 2006; Holtzman et al., 2006). Findings revealed that trigeminal brainstem nuclei interpolaris, oralis, and principalis project predominantly ipsilateral to cerebellar regions. Taken together, cerebellar cortices receive mostly ipsilateral and to a lower extend, bilateral fibers from several trigeminal brainstem nuclei (detailed summarized by Dietrichs and Walberg, 1987). Recent work by Borsook et al. (2008) provides an overview of 28 studies with cerebellar activation in acute experimental pain using fMRI and PET. Bilateral activity is described in 15, ipsilateral activity in 10, and contralateral activity in 3 of them. This is an astonishing observation as most of the reviewed investigations stimulated the upper extremities unilaterally. Considering the anatomical perspective provided by animal research, one would expect a predominantly ipsilateral and to a smaller extent, bilateral activation. They also summarize own research on investigating specifically noxious

and non-noxious thermal heat and brush stimuli applied to the maxillary division of the face in healthy and neuropathic pain patients. Summarized, noxious heat evoked predominantly contralateral activation in both groups, while brush evoked more ipsilateral cerebellar activity. Based on their observations a "dichotomy of innocuous stimuli/sensorimotor cerebellum activation versus noxious experience/cognitive/limbic cerebellum activation" was suggested.

Our data show a right-lateralized effect in both, anterior and posterior cerebellum as well as in the parahippocampus. Schmahmann and Pandya (1997) as well as Manto (2006) describe outputs to numerous (limbic) structures, among them; hippocampal complex, amygdala, thalamic nuclei, hypothalamus, and the periaqueductal gray. Based on these connections, the cerebellum has also been called "modulator of different neurologic functions," thus directly influencing sensory, but also emotional and cognitive processing (Allen et al., 2005; Ito, 2008).

The role of the basal ganglia in processing nociceptive information is still debated despite their robustly observed involvement shown in human studies (Coghill et al., 1999, 2001; Apkarian et al., 2005) as well as in animal research (Chudler, 1998). Neuroanatomical evidence reveals afferents from several subdivisions of the cerebral cortex (including neocortical and cingulate cortex), thalamic nuclei, cerebellum, the amygdala, parabrachial area, and dorsal raphe nucleus (Chudler and Dong, 1995; Downar et al., 2003). Although the main role of the basal ganglia is often related to sensorimotor integration and thus adaptation of motor responses to noxious stimuli, their involvement in other dimensions of pain processing cannot be excluded. The review of Chudler and Dong (1995) provides strong evidence for a functional involvement of the basal ganglia in both, direct innocuous and noxious somatosensory processing. Supporting this finding, Coghill et al. (1999) pointed out the role of the putamen and globus pallidus (bilateral) in processing of human pain intensity and Scott et al. (2006) linked the role of the putamen to anticipatory mechanisms. Publications of several other investigations suggest cerebellar and basal ganglia processing to depend on cognitive functions (Akshoomoff and Courchesne, 1992; Schmahmann and Pandya, 1997; Schmahmann and Caplan, 2006). However, based on present literature no evidence emerges regarding lateralization of cognitive functions in these areas. Therefore,

we do not assume that left-lateralization found in our data indicates cognitive involvement, but rather reveals motor functions, many of which are known to be lateralized to the motor dominant hemisphere. This interpretation is up for debate as two previous studies revealed certain aspects of hemispheric dominance to be independent of handedness for noxious and non-noxious somatosensory stimulation (Jung et al., 2003; Schlereth et al., 2003).

Focusing on significantly activated cingulate cortex subdivisions (PCC, pMCC, aMCC, pACC, and sACC) we found a left hemispheric lateralization in the pACC and a trend toward left-lateralization in the aMCC, but no lateralization in the more posterior divisions. Current literature indicates that pACC is associated with engaging in positively valenced events and is linked with the amygdala's lateral basal and accessory basal nuclei, whereas the aMCC contains the rostral cingulate motor area (Vogt, 2005). Based on their findings, Büchel et al. (2002) concluded that a main function of the ACC's subdivisions is to integrate a wide range of pain relevant information and to generate adequate responses. However, considering pain related investigations, distinct lateralization aspects of ACC subdivisions have to date not been in the focus of interest. In line with its functional attributes (selection of adequate reactions), the aMCC activation pattern found in our study points toward involvement in motor components of nociception, as seen for cerebellum and putamen (Vogt, 2005).

The left-lateralization effect noticed in the supramarginal area (BA 40) may also relate to a functional role of this structure in sensorimotor integration (Serrien et al., 2006), or a specialization for the detection of behaviorally relevant stimuli (Corbetta and Shulman, 2002).

Even if the stimuli may not be interpreted by subjects as potentially dangerous, pain is inherently salient (Legrain et al., 2009). Conform to Farrer et al. (2008) we favor an interpretation that the left lateralized activation within the supramarginal area is related to the analysis and integration of body-related nociceptive sensations in contrast to right-lateralized parietal cortex activity which is thought to mediate the analysis and integration of body-related visual and painless somatosensory information.

With respect to the finding that parahippocampus shows predominantly right sided BOLD responses to dental nociceptive stimuli, the function of this structure may also be described

in the context of novelty detection theories, as suggested before by Bingel et al. (2002) and Ploghaus et al. (2000) and corroborated by Strange and Dolan (2006) with fear related stimuli.

STRUCTURES WITH PREDOMINANT CONTRALATERAL ACTIVATION

We found evidence in five brain areas that reveal activation dominance contralateral to the side of stimulation: SI, thalamus, posterior insula, amygdala, and subcentral area (BA 43). Subcentral area additionally demonstrates hemispheric lateralization and will be discussed later.

Contralateral activation is closely linked to somatotopic encoding. Yet, unresolved questions exist as to lateralization aspects in cortical structures like SI, SII, thalamus, and posterior insula. To address this topic was one of the aims of the present study. Previously, Bingel et al. (2003) have investigated lateralized brain activity in response to noxious stimuli in SI, SII, insula, and thalamus and found contralateral bias in all these four areas. Although stimulation of either hand evoked bilateral activation of anterior and posterior insular regions, a contralaterally biased response was found for the posterior parts of the insula bordering SII. Similar findings were reported by Brooks et al. (2002) who applied noxious thermal stimuli to both hands. Again, activation in insular posterior parts was dependent on the site of stimulation, whereas this dependency was absent in more anterior insular areas and SII. Interestingly, activation was absent in thalamus and SI. If activation in the thalamus is reported, then mostly contralateral but also often bilateral (Peyron et al., 2000) although, more recently, Kulkarni et al. (2005) reported ipsilateral, but no contralateral thalamus activity.

Our electric dental stimulation data show robustly that SI is activated bilaterally with a significant predominance contralateral to the stimulus application side. The same findings hold true for thalamus, and posterior insular cortex (Figure 3). We thus confirm the functional role of these cortical areas in topographic stimulus encoding.

Possibly, lateralized activation of areas could be caused by evasive or protective motor action dependent on the site of stimulation. However, this unlikely explains the present data, since withdrawal and orientation responses have been shown to predominantly

activate cingulate cortex subdivisions (Vogt, 2005; Peyron et al., 2007) and cerebellum (Dimitrova et al., 2003) but not SI, thalamus, posterior insula, amygdala, or subcentral area (BA 43).

The amygdala's involvement in various forms of conditioned hypoalgesia and analgesia has been well established in several animal studies (e.g., Crown et al., 2000; Neugebauer and Li, 2002, 2003; Neugebauer et al., 2004). Lesion studies, specifically of the latero-capsular amygdaloid nucleus (also termed "nociceptive amygdala") demonstrated reduced or completely abolished conditioned behavior (Watkins et al., 1998). Inconsistent amygdala activation in response to nociceptive and other aversive stimuli in humans is frequently reported (Baas et al., 2004; Phan et al., 2004; Rempel-Clower, 2007; Tracey and Mantyh, 2007). Why amygdala activation appears robustly in response to noxious dental stimulation in comparison to stimulation of other body parts (Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005) can only be speculated. One possible explanation is that the amygdala has proven relevant for emotional conditioning (Büchel et al., 1999; Büchel and Dolan, 2000; Cardinal et al., 2002) and thus, a unique emotional salience of dental pain could explain our findings. However, it must be noted that the emotional value of the applied stimuli has not been directly controlled for. Stimulus conditioning and (missing) previous dental pain experiences could both contribute to an assumed peculiarity of dental pain. Alternatively dental pain may involve different processing pathways (trigeminal versus spinal). Future investigations need to further elucidate this topic.

Lateralization of amygdala activation shows an inconsistent picture. Among human neuroimaging studies, none described a clearly lateralized activation dependent on the stimulation side (e.g., Bingel et al., 2002; Bornhovd et al., 2002). The present data show that BOLD signal in the amygdala is stronger contralateral than ipsilateral to the side of stimulation. To the best of our knowledge, this has previously not been shown in pain studies nor in investigations on emotion. Regarding the latter, Baas et al. (2004) pointed out that there is no stimulation side dependent amygdala lateralization effect across 54 studies analyzed by them. One has of course to consider different paradigms and also different statistical approaches which hamper an adequate conclusion so far. Our approach of analyzing mean activations by a RM-ANOVA provides some evidence toward possible

somatotopic related encoding properties. Previous studies may have missed a lateralization effect in the amygdala due to less salient stimuli and/or bigger voxel sizes (introducing greater partial volume effects and hence reduced statistical power).

Interestingly, contrary to previous reports our data do not indicate lateralization of brainstem activity. We propose that this is due to methodological reasons. Without applying special imaging techniques, brainstem activity is often severely masked by movement artifacts stemming from pulsation movements of the A. carotis. Correction of these artifacts involves, e.g., cardiac triggering, which we did not apply for sake of greater power in the remaining regions. Methods to deal with physiological artifacts *post hoc* (see e.g., Harvey et al., 2008) were also not applicable due to missing cardiac and respiratory information. Thus we argue that brainstem effects are likely to be missed in our study which should not give rise to suspicion regarding the effects found.

### STRUCTURES SHOWING HEMISPHERIC DOMINANCE AND PREDOMINANT CONTRALATERAL ACTIVATION

The subcentral area (BA 43) shows significant lateralization to one hemisphere (main effect "hemisphere") and also significant enhanced activation contralateral to the stimulus. Interestingly, this area is not frequently reported in pain studies. Subcentral area (BA 43) is located at the ventral end of the pre/postcentral gyri and the bank of the lateral sulcus and also delineated as SII. Its rostral and caudal borders are neighbored by both, the anterior and posterior subcentral sulci. Its distinction from surrounding areas is based on its specific cytoarchitectonic features already observed by Brodman (Eickhoff et al., 2006 and 2007).

Only few human studies explicitly reported lateralized activation within BA 43 in response to noxious stimulation. Becerra et al. (2001) noted right-lateralized activation in BA 43 in response to noxious thermal hand stimulation, but this result was not addressed in the discussion. Focussing on idiopathic chronic low back pain, Giesecke et al. (2004) found bilateral activation in BA 43 and discussed it as being part of the secondary somatosensory cortex. In a simultaneous EEG-fMRI investigation, Christmann et al. (2007) reported bilateral activation within BA 43 and also delineated it as being part of SII. However, in none of these studies, activity within BA 43 was further interpreted by the authors.

The present data showed a strong hemodynamic response within BA 43, with a significant interaction effect between stimulated tooth and hemisphere (activity is predominantly contralateral to the stimulus) as well as a main effect towards the right hemisphere (Table 3 and Fig. 3). This distinct right-lateralized activation is eye-catching and the present data may shed new light on the role of this structure, since the activation pattern is quite different from other parts of SII. Strong anatomical connections between the subcentral area and pre-motor cortices, as well as posterior parietal area (Cipolloni and Pandya, 1999) place the subcentral area (BA 43) in an ideal position for multimodal sensorimotor integration. Such a role has long been suggested for mammals (Krubitzer, 1996) and more recently for humans (Disbrow et al., 2000).

Although our results point towards a specialized somatosensory encoding function with a possible role in sensorimotor integration, it may be premature to speculate on the specific role of BA 43 within the pain circuitry.

## STUDY LIMITATIONS

A full understanding of brain activations in response to painful stimuli is inherently limited by the complexity of the multidimensional pain experience. Some brain activity patterns may not necessarily be directly involved in pain processing, but rather relate to aspects of alertness and/or orientation responses. Namely parieto-occipital activation clusters may be interpreted in this way. The human pain experience implies orientation toward pain and toward options to relieve it. Some brain activity may thus not be directly linked to the pain experience itself. Furthermore, as the intensities of all stimuli were above the pain threshold, purely somatosensory processes cannot be controlled for and thus it cannot be excluded that some brain activities may reflect somatosensory aspects of the stimulation. Finally, although all subjects located their pain to the stimulation tooth, we are unable to report on the fiber subpopulations involved in pain transmission.

## CONCLUSIONS

Electrically evoked dental pain activates cortical areas typically described in spinal pain studies. Yet, robust activation can be observed in additional areas, namely the amygdala. Besides previously known lateralization effects, hemispheric lateralization irrespective of

side of stimulation were observed in subdivisions of the ACC (aMCC and pACC). Predominant contralateral activation in the posterior insular cortex and the amygdala points towards their possible involvement in somatotopic encoding of noxious stimuli, in addition to other, previously described functions.

## CONFLICT OF INTEREST STATEMENT

All authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.


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## SUPPLEMENTAL MATERIAL

Supplementary table 1: Complete list of local maxima within clusters activated for the contrast stimulation versus baseline (as illustrated in Fig. 2). As there are very large clusters, anatomical descriptions are related only to the maximally activated voxel within each cluster.

| Anatomical Description | Cluster Size | Voxel p (FWE-cor) | MNI Coordinates (max T Voxel) | Voxel T (max T) |
|---|---|---|---|---|
| Postcentral Gyrus | 27749 | 0.000 | 14.74 | -38 -36 54 |
| | | 0.000 | 14.55 | -18 14 -2 |
| | | 0.000 | 14.21 | -40 -28 56 |
| | | 0.000 | 13.92 | -38 -32 62 |
| | | 0.000 | 13.16 | 50 -30 52 |
| | | 0.000 | 13.13 | 38 -34 48 |
| | | 0.000 | 12.85 | 44 -62 4 |
| | | 0.000 | 12.65 | -34 -44 56 |
| | | 0.000 | 12.52 | -34 -42 52 |
| | | 0.000 | 12.5 | 42 -36 54 |
| | | 0.000 | 12.43 | -42 -32 46 |
| | | 0.000 | 12.34 | -46 12 -10 |

| | | | | |
|---|---|---|---|---|
| | | 0.000 | 12.25 | -46 -20 58 |
| | | 0.000 | 12.05 | -56 -28 52 |
| | | 0.000 | 11.75 | -42 -28 18 |
| | | 0.000 | 11.7 | -58 -22 14 |
| | | 0.000 | 11.56 | 58 12 -8 |
| | | 0.000 | 11.55 | -52 -22 54 |
| | | 0.000 | 11.55 | 40 2 -18 |
| | | 0.000 | 11.46 | -54 -26 16 |
| | | 0.000 | 11.44 | -40 -36 42 |
| | | 0.000 | 11.43 | 54 -22 44 |
| | | 0.000 | 11.43 | 34 -8 64 |
| | | 0.000 | 11.38 | 36 -18 66 |
| | | 0.000 | 11.18 | -40 0 -12 |
| | | 0.000 | 11.15 | 50 16 -14 |
| | | 0.000 | 11.04 | -10 -20 8 |
| | | 0.000 | 11.02 | -52 4 -6 |
| | | 0.000 | 10.98 | -56 6 -2 |
| | | 0.000 | 10.85 | -46 4 -4 |
| | | 0.000 | 10.84 | 36 -46 54 |
| | | 0.000 | 10.78 | -22 -66 62 |
| Posterior Cingulate | 3304 | 0.000 | 13.16 | -4 -32 26 |
| | | 0.000 | 11.68 | 0 6 48 |
| | | 0.000 | 11.35 | -2 -14 56 |
| | | 0.000 | 11.29 | -2 -2 48 |
| | | 0.000 | 11.09 | -2 16 38 |
| | | 0.000 | 10.82 | 2 -8 46 |
| | | 0.000 | 10.24 | -8 -28 44 |

| | | | | |
|---|---|---|---|---|
| | | 0.000 | 9.98 | 0 -26 54 |
| | | 0.000 | 9.96 | -2 -6 56 |
| | | 0.000 | 9.95 | -2 18 46 |
| | | 0.000 | 9.11 | 2 -22 44 |
| | | 0.000 | 8.96 | 2 -2 66 |
| | | 0.000 | 8.87 | 4 -2 38 |
| | | 0.000 | 8.82 | 10 22 28 |
| | | 0.000 | 8.75 | -10 18 30 |
| | | 0.000 | 8.2 | -2 30 18 |
| | | 0.000 | 7.74 | 6 -4 30 |
| | | 0.000 | 7.58 | 8 16 64 |
| | | 0.000 | 7.31 | 4 -20 28 |
| | | 0.000 | 6.92 | -4 26 38 |
| | | 0.000 | 6.86 | 2 -14 28 |
| | | 0.000 | 6.48 | -2 38 10 |
| | | 0.003 | 6.02 | 10 4 40 |
| Midbrain | 99 | 0.000 | 10.17 | 2 -16 -14 |
| | | 0.000 | 7.4 | 4 -26 -28 |
| | | 0.000 | 7.2 | 0 -18 -20 |
| Cerebellum posterior lobe | 117 | 0.000 | 9.6 | 14 -76 -48 |
| Medulla | 72 | 0.000 | 8.07 | -2 -34 -50 |
| | | 0.000 | 6.48 | -6 -34 -42 |
| Inferior Frontal Gyrus | 37 | 0.000 | 7.53 | 52 44 4 |
| | | 0.001 | 6.25 | 54 40 -2 |
| Cerebellum posterior lobe | 26 | 0.000 | 7.19 | 32 -80 -32 |
| Temporal inferior Lobe | 31 | 0.000 | 6.89 | -44 -42 -28 |
| | | 0.000 | 6.51 | -38 -48 -24 |

| | | | | |
|---|---|---|---|---|
| | | 0.002 | 6.11 | -48 -42 -26 |
| Cingulate Gyrus | 13 | 0.000 | 6.79 | 14 -28 36 |
| Occipital Lobe (Lingual) | 55 | 0.000 | 6.69 | 2 -68 4 |
| | | 0.000 | 6.53 | 6 -64 0 |
| Parietal Lobe (Precuneus) | 17 | 0.000 | 6.62 | -6 -52 52 |
| | | 0.001 | 6.25 | -2 -58 52 |
| Inferior Parietal Lobe (Supramarginal) | 11 | 0.001 | 6.2 | 68 -36 26 |

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## 2.2 STUDY II:

### BRAIN ACTIVATION INDUCED BY DENTINE HYPERSENSITIVITY PAIN - AN FMRI STUDY.

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ABSTRACT

Aim: Dentine hypersensitivity (DH) is characterized by a short, sharp pain arising from exposed dentin. Most published literature reports on peripheral neural aspects of this pain condition. The current investigation focused on differential cerebral activity elicited by painful and painless stimulation of sensitive and insensitive teeth.

Materials and Methods: Five graded stimulus strengths were randomly applied by means of a multi-injector air jet delivery system, each followed by an individual rating of perceived stimulus intensity. Brain activity was analyzed by functional magnetic resonance imaging (fMRI).

Results: Stimulation of sensitive teeth induced significant activation in the thalamus, somatosensory cortices (SI & SII), anterior, middle and posterior insular cortices, anterior mid-cingulate cortex, perigenual anterior cingulate cortex and frontal regions (BA10 and BA46). Differential responses to DH and painless perceptions were observed in the anterior insula and anterior mid cingulate cortex.

Conclusion: For the first time this fMRI study demonstrates the feasibility to investigate cerebral processes related to DH evoked by natural (air) stimuli. Our neuroimaging data additionally provide evidence that differential activity in the anterior Insula (aIC) and anterior midcingulate cortex (aMCC) may represent clinically relevant pain experienced by DH patients.

CLINICAL RELEVANCE

Scientific rationale for the study: DH can considerably impact quality of life. Pain research revealed that besides brain areas coding somatosensory information, regions for emotional and cognitive-behavioral signal processing are additionally activated during nociception. Brain activation in response to “natural” DH provoking stimuli was never investigated and was therefore this study’s aim.

Principal findings: The present feasibility study provides the first functional neuroimaging data on human brain activity in response to graded air stimuli applied to sensitive and insensitive teeth.

Practical implications: Our new experimental approach is likely to improve our understanding of the neurobiology underlying DH beyond peripheral processes.

## INTRODUCTION

DH is a common clinical problem often related to periodontal disease (Rees & Addy, 2002, Que et al., 2010). DH meets all criteria to be classified as a true pain condition (Curro, 1990) involving sensory, cognitive, emotional and motivational dimensions. Various peripheral stimuli to exposed dentin (thermal, evaporative, tactile, osmotic or chemical) provoke the characteristic short, sharp pain (Dowell & Addy, 1983). Peripheral nociceptive processes underlying DH are thought to involve temperature or pressure alterations in open dental tubules which provoke fluid movements and that in turn result in stimulation of pulpal nerves, namely nociceptive A-delta and C-fibers (Orchardson & Cadden, 2001). Due to their myelin sheet, A-delta fibers conduct action potentials faster than unmyelinated C fibers and are therefore held responsible for generating the characteristic DH pain (Abd-Elmeguid & Yu, 2009). Although animal studies much improved our understanding of brain stem mechanisms involved in orofacial pain (Sessle, 2000) no information is currently available on human cerebral processes induced by DH. Insights into pain related human brain function is made feasible by modern neuroscientific techniques including functional magnetic resonance imaging (fMRI). Neuroimaging methods revealed that a group of specific brain areas, known as the pain or nociceptive matrix, form a modular network that is preferentially activated by painful stimuli. Following pain application to territories innervated by spinal nerves, the primary (SI) and secondary (SII) somatosensory cortices, subdivisions of the cingulate cortex, the insular cortex, thalamus, cerebellum and frontal regions showed altered neural activity (Peyron et al., 2000, Apkarian et al., 2005). The same network was also consistently activated by painful tooth stimulation elicited by electric current (Ettlin et al., 2009, Weigelt et al., 2010, Brügger et al., 2011), and less consistently by painless dental stimuli (Ettlin et al., 2004, Habre-Hallage et al., 2010). Although toothache induction by electric current has merits as an experimental model, air blast application to sensitive teeth better mimics the clinical pain experienced by patients suffering from DH. Prior investigations indeed demonstrated that a single air blast to hypersensitive teeth is an appropriate stimulus (Ide et al. 2001, Yilmaz et al., 2011). However, these studies used a steady air flow.

The current report was planned as feasibility study and aimed at elucidating cortical processes due to graded air blasts application to sensitive and insensitive teeth. We firstly expected to evoke activations associated with the pain matrix. Secondly, we hypothesized to find cortical substrates which show a differentiation between sensitive and insensitive teeth stimulation. To the best of our knowledge, this is the first report using fMRI for investigating DH.

## MATERIALS & METHODS

### RECRUITMENT AND SENSITIVE TOOTH ASSESSMENT

From 71 potential subjects answering an online questionnaire upon having read a web announcement, 26 subjects were selected and invited for a screening visit. During this visit, the sensitive tooth and a healthy insensitive tooth were clinically and radiographically evaluated by a dentist. Only incisors, canines and premolars were evaluated since molars were not suitable for installation of the air delivery tubes (Figure 1). Sensitivity to air was tested by a triple air syringe commonly used in dentistry. Subjects were instructed to report stimulus perceptions by means of a horizontal 0-10 numerical rating scale (NRS) with 0 labeled as “no pain” and 10 as “worst imaginable pain”. For each tooth, a rating of at least 5 was required to be classified as sensitive. If several sensitive teeth were diagnosed, the tooth with the most intense pain perception was chosen. Insensitive teeth contralateral to the sensitive tooth were tested for air blast insensitivity. 16 subjects did not fulfill the inclusion requirements: 13 of them had no insensitive tooth on the opposite side and 3 subjects experienced sensitivity in molar teeth only. The final DH study group consisted of 10 subjects (age 21 – 55, mean 29.7, eight females). The sensitive tooth was located in the maxillary jaw in 9 of 10 subjects. In five subjects the sensitive tooth was located on the right side.

The study was approved by the local ethics committee of the University of Zurich and conducted according to the guidelines of the Declaration of Helsinki for treatment of experimental human subjects. Subjects were financially compensated.

### EXPERIMENTAL MATERIAL

Blu-Mousse (Thixotropic Vinyl Polysiloxane, Edgewood, MD, USA) impressions were taken from the subject's dentition. 6mm diameter holes were drilled at the labial gingival margin of

test teeth. Two clear polyurethane tubes (Festo AG, Dietikon, Switzerland) of 4 mm inner diameter for air stimulation were permanently mounted into the holes of the impression with blu-mousse. For outward flow of the applied air little grooves were drilled beside the tube holding holes (Figure 1).

A modified portable version of the air puff delivery system previously described (Megias-Alguacil et al., 2008) was used for tooth stimulation (Figure 1). This system is capable of operating in a magnetic resonance imaging environment and enables application of graded air streams with flow rates starting at 1 l/min (barely noticeable) to 20 l/min which corresponds to the air stream exiting from a typical triple air syringe.

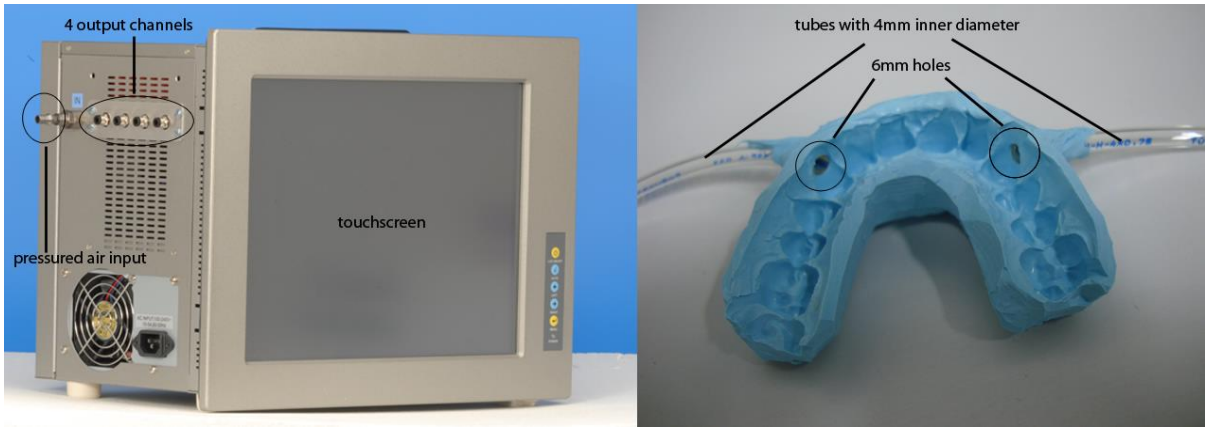



Figure 1. Left: MRI compatible multi-injector gas jet delivery system with touch screen. Right: Individual dental polysiloxane impression

## PSYCHOPHYSICAL EXAMINATION

Between one and two weeks prior to the MR experiment, subjects received extensive training during a psychophysical test session which served to familiarize subjects with the stimulation paradigm. For determination of stimulus perception threshold, subjects were seated upright in a dental chair and comfortable fit of the stimulation tube holding impression was checked. In particular, care was taken that the soft splint did not evoke any pain or discomfort. Air blast stimuli of 1s duration were applied at randomized inter-stimulus intervals (ISI) between 7.5-12.5s. Using a staircase method, the sensory detection threshold (SDT = defined as the lowest flow rate at which the volunteer sensed an air puff) was determined, starting at a flow rate of 1 l/min (system inherent lower limit), with subsequent 1 l/min increments. Pain detection threshold (PDT = the lowest flow rate that

was perceived as just painful) and pain tolerance threshold (PTT = the maximum air flow rate that the subject would freely tolerate) were determined by further stepwise increases of flow rates. The threshold detection procedure was repeated three times with 5 minute breaks between each series. SDT, PDT and PTT were calculated as the mean of three repetitive measurements. All stimuli were controlled by a computer and neither the test persons nor the investigator viewed the computer screen to reduce bias in the psychophysical assessment procedure. The five stimulus strengths for the fMRI protocol were calculated as follows: PDT-40%, PDT-10%, PDT+20%, PDT+40% and PDT+60% (Figure 2). The same stimulus strengths were also applied to the insensitive tooth.

Once thresholds were determined, a psychophysical testing session was performed in order to familiarize subjects with the fMRI test protocol. The scanner environment was simulated by dimming room light and subjects were placed in supine position. They were given a headset playing an fMRI-EPI-sequence audiofile and were asked to wear video goggles displaying a computerized visual rating scale (coVRS) with 12 marks. The left anchor (first mark) was labeled “no sensation”, the 4th mark “pain threshold” and the right anchor (12th mark) “worst imaginable pain” (Figure 2). This approach enabled subjects to rate the perceived intensity of painless and painful stimuli using the same scale. Subjects were instructed to concentrate explicitly on the intensity of the perceived stimulus. The coVRS appeared one second after stimulus onset and was shown for six seconds during which subjects moved the lever of a MR compatible potentiometer. The position of this lever was linearly transformed into the position of a mark on the rating scale. The stimulation protocol consisted of 50 stimuli (10 stimuli/strength) applied in random order with a randomized ISI between 7.5 and 12.5 seconds in order to minimize anticipation and to optimize peri-stimulus fMRI sampling times (Figure 2). After disappearance of the rating scale, a fixation cross was displayed until the next rating scale appeared.

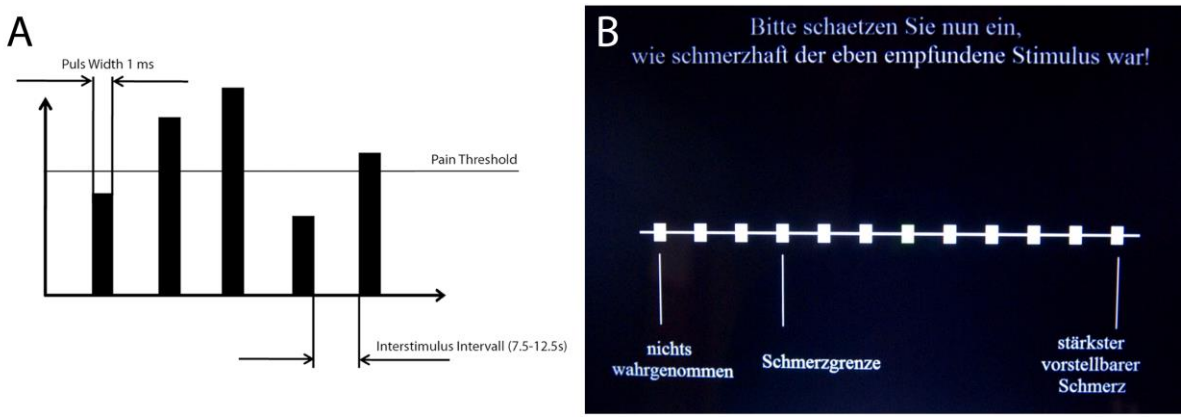



Figure 2. A. Schematic of the fMRI paradigm. Stimulus duration was set to 1s, interstimulus intervals were kept between 7.5 and 12.5 seconds. Strength of the stimuli were PPT - 40%, PPT-10%, PPT + 20%, PPT + 40% and PPT + 60%. The different strengths have then been applied randomly and subjects were required to rate every stimulus with respect to their perceived intensity by means of a MR compatible rating scale. B. Illustrates the computerized visual rating scale (coVRS) the way it had been projected after every stimulus for 6 seconds. Green color indicated the rectangle moved by the subject. Left; no perception (nichts wahrgenommen), the fourth rectangle; pain threshold (Schmerzgrenze), right; worst imaginable pain (stärkster vorstellbarer Schmerz). Important to note: subjects were trained prior to the fMRI experiment to handle correctly the coVRS and questions/uncertainties were answered. However, all subjects quickly understood the use of the scale.

## FMRI DATA ACQUISITION

Within two weeks after psychophysical testing, subjects underwent the fMRI protocol in a Philips 3-Tesla Achieva System (Philips Medical System, Best, The Netherlands). The protocol started by retesting individual thresholds (SPT, PDT and PTT). If either SDT or PDT deviated more than 20% from the value assessed during the previous psychophysical test session, subjects were excluded from further participation. Since several investigations indicate a diurnal variation of pain perception (Fillingim & Ness, 2000; Koch & Raschka, 2004), this investigation took place at the same daytime as the psychophysical examination. Subjects underwent the same stimulation protocol as performed during psychophysical examination, except that headphones were replaced by earplugs and real fMRI scans were acquired.

For functional scanning, a blood oxygen level dependent (BOLD) sensitive single-shot gradient echo planar imaging sequence was used to acquire 33 axial slices, covering the entire cerebrum and cerebellum, using an 8 channel receive-only head coil. Parameters: echo time = 30 ms, flip angle = 75 degrees, repetition time = 2500 ms, slice thickness = 4 mm, inter-slice gap = 0 mm, field of view = 230 mm and matrix size in plane = 128 x 128, resulting in a voxel size of 1.72 x 1.72 x 4 $mm^3$. Three dummy scans were first acquired and

discarded to reach steady state magnetization. Additionally, 180 high-resolution T1 weighted axial slices (spoiled gradient echo) were acquired with TR = 20ms, flip angle = 20°, voxel size = 0.98 x 0.98 x 1.02 $mm^3$, FOV = 22 cm, matrix = 224 x 187, which were used as an underlay for individual functional maps and for obvious neurological disorders.

After the experimental protocol, participants were asked whether they had perceived the stimulation in the test tooth only or also in adjacent tissue.

### DATA ANALYSIS

For the current report, we focused on the stimulus strengths 3-5 of the sensitive tooth (painful) and insensitive tooth (painless) to investigate specific cortical underpinnings of the painful perceptions of DH in comparison to the painless perceptions elicited on the insensitive tooth with identical stimuli. Psychophysical data, i.e., the relation between the physical stimulus strength and the subjective intensity rating, as well as region of interest (ROI) data, i.e., the relation between the physical stimulus strength and corresponding signal change in each ROI, have been analyzed using SPSS 17 (SPSS Inc, Chicago, Illinois 60606, USA).

SPM5 (http://www.fil.ion.ucl.ac.uk/spm) software package running on MATLAB R2008b (Mathworks, Natiek, USA) was used for functional voxel-by-voxel analysis. In a first step, spatial realignment to the first image in the series as reference was performed and it was assured that detected movement did not exceed 1.5 mm (translational) or 1° (rotational) in relation to the first image. For studying group effects, data were normalized to the MNI template brain (Evans et al., 1992) followed by smoothing with a Gaussian kernel of 6 mm (FWHM).

Image analysis to reveal significant changes in cortical activity due to the three painful stimulus strengths of the sensitive tooth and the three non-painful strengths of the insensitive tooth (conditions) was performed on each subject's data by means of individual (1st level) general linear models using the hemodynamic response function implemented in SPM5. Statistical parametric maps were then calculated, yielding beta estimates of the model fit for each subject and condition.

We defined a ROI mask in the voxel-by-voxel analysis, comprising several brain regions involved in pain processing (Apkarian et al., 2005). Since DH is classified as a true pain condition we expected mainly activity among these regions. The primary and secondary somatosensory cortex (SI & SII), insular cortex, anterior cingulate cortex (ACC), thalamus and prefrontal cortex (PFC) were taken from the SPM tool "WFU-Pickatlas" (Lancaster et al., 1997; Lancaster et al., 2000) and the "SPM Anatomy Toolbox" (Eickhoff et al., 2005): In the voxel-by-voxel analysis, the ROI mask was applied as an explicit mask, which limits the investigated voxel space masking region. Average group statistical map was then calculated (second level) in a random effects model, using one-sample t-tests, testing the BOLD response to each painful stimulus strength against the null hypothesis of no related signal change. Resulting voxel T-values were color-coded and superimposed onto the MNI single-subject-T1 brain (Figure 4)

For more detailed investigation of the trigeminal nociceptive system, we calculated the mean activation in predefined anatomical regions of interest (ROI). For this purpose, the insula regions were divided into three parts, namely in an anterior (aIC), middle (mIC) and posterior (pIC) part, according to several reports which suggest a complex anatomical (Varnavas & Grand, 1999) and functional (Brooks et al., 2002; Brooks et al., 2005) fragmentation within this particular brain area. The investigated cingulate cortex regions consisted of a subgenual part (sACC), a perigenual part (pgACC) and more posterior part, namely the anterior mid cingulate cortex (aMCC), after the classification of Vogt (2005). The secondary somatosensory cortex (SII) was delineated into four subregions OP1 – OP4 based on Eickhoff et al. (2006). Finally, frontopolar (BA10) and frontomedial (BA46) areas constituted the prefrontal cortex.

The mean activation within each ROI, determined by the individual mean beta values, was calculated for each of the three stimulus strengths across both teeth. A repeated measures ANOVA was then calculated for all ROIs with tooth (sensitive / insensitive) as within-subject factor.

## RESULTS

### PSYCHOPHYSICS

Due to the brief after-scanning interview we were able to assure that all subjects felt the sensation at the stimulated tooth only. In all subjects the highest applied stimulus strength was below PTT. Subjects reported no unpleasant or otherwise disturbing perceptions due to the inserted splint. In addition, they felt no lingering sensation after the stimulation, indicating that no tissue sensitization had been induced due to the experimental setup. Furthermore, no subjects had to be excluded due to excessive deviations from SDT, PDT and PTT values of the psychophysical examination.

Subjective mean ratings of the respective stimulus strengths during the scanning session show clearly that the two lowest stimulus strengths applied on the sensitive tooth were rated as non-painful whereas stimulus strengths 3-5 were rated as painful. As expected, stimulations of the insensitive tooth were always rated as non-painful (Figure 3).

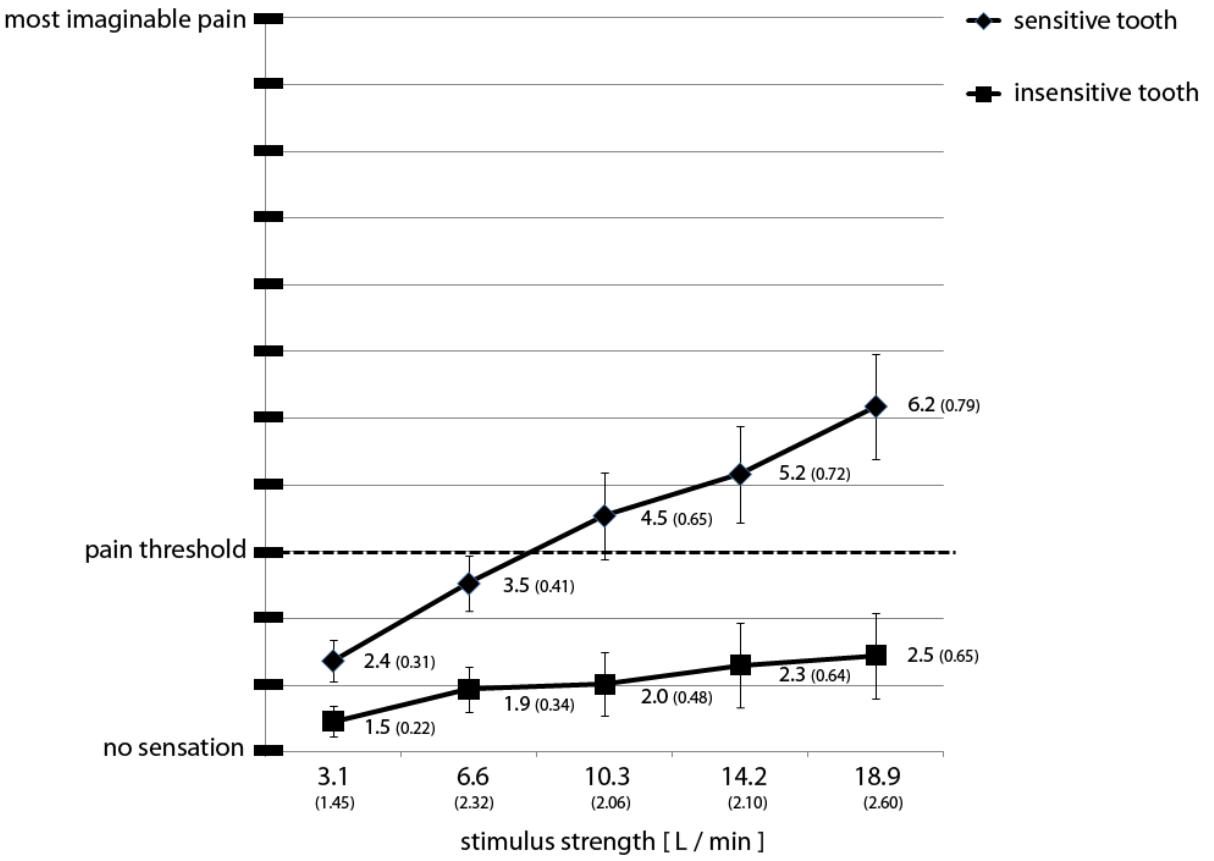



Figure 3: Group mean stimulus strength in l / min and corresponding mean coVRS ratings (with standard errors of the mean in brackets and graphically shown as T-bars) during the fMRI stimulation experiment. The pain threshold is illustrated by the dashed line.

## BRAIN ACTIVATION PATTERNS

Painful stimulation of the sensitive tooth induced significant activation in somatosensory cortices (SI & SII), anterior, middle and posterior insular cortices, pgACC and aMCC, Thalamus as well as frontal regions (BA10 and BA46, figure 4). Anatomical description, size of biggest cluster in the respective ROI and coordinates of the maximum p and t- values for the sensitive tooth stimulation are listed in table 1.

| **Brain Region (left \| right)** | **Cluster size (N voxel)** | **local maxima (p-values, FWE-corrected for multiple comparisons)** | **local maxima (T-values)** | **local maxima (MNI coordinates)** |
|---|---|---|---|---|
| SI \| left | 738 | 0.000 | 10.77 | -54 -32 54 |
| SI \| right | 42 | 0.000 | 8.29 | 60 -16 44 |
| SII \| left | 137 | 0.000 | 7.99 | -56 -26 14 |
| SII \| right | 72 | 0.000 | 10.63 | 62 -14 10 |
| aIC \| left | 287 | 0.000 | 9.43 | -30 22 -2 |
| aIC \| right | 254 | 0.000 | 11.63 | 34 26 -2 |
| mIC \| left | 102 | 0.000 | 8.60 | -44 10 -8 |
| mIC \| right | 165 | 0.000 | 11.59 | 44 10 -2 |
| pIC \| left | 45 | 0.003 | 4.75 | -42 - 12 2 |
| pIC \| right | 0 | - | - | - |
| Thalamus \| left | 102 | 0.000 | 8.72 | -14 -20 2 |

| | | | | |
|---|---|---|---|---|
| Thalamus \| right | 3 | 0.000 | 6.21 | 12 -12 2 |
| pgACC \| left | 14 | 0.000 | 6.66 | -8 32 18 |
| pgACC \| right | 6 | 0.000 | 6.49 | 4 32 34 |
| aMCC \| left | 298 | 0.000 | 8.21 | -2 30 32 |
| aMCC \| right | 389 | 0.000 | 10.58 | 6 26 38 |
| BA 10 \| left | 4 | 0.000 | 6.81 | -30 44 30 |
| BA 10 \| right | 27 | 0.000 | 7.94 | 38 40 22 |
| BA 46 \| left | 11 | 0.000 | 6.59 | -40 34 20 |
| BA 46 \| right | 41 | 0.000 | 8.48 | 44 38 20 |

Table 1. Peak activations of brain areas during painful stimulation of the sensitive tooth vs baseline.

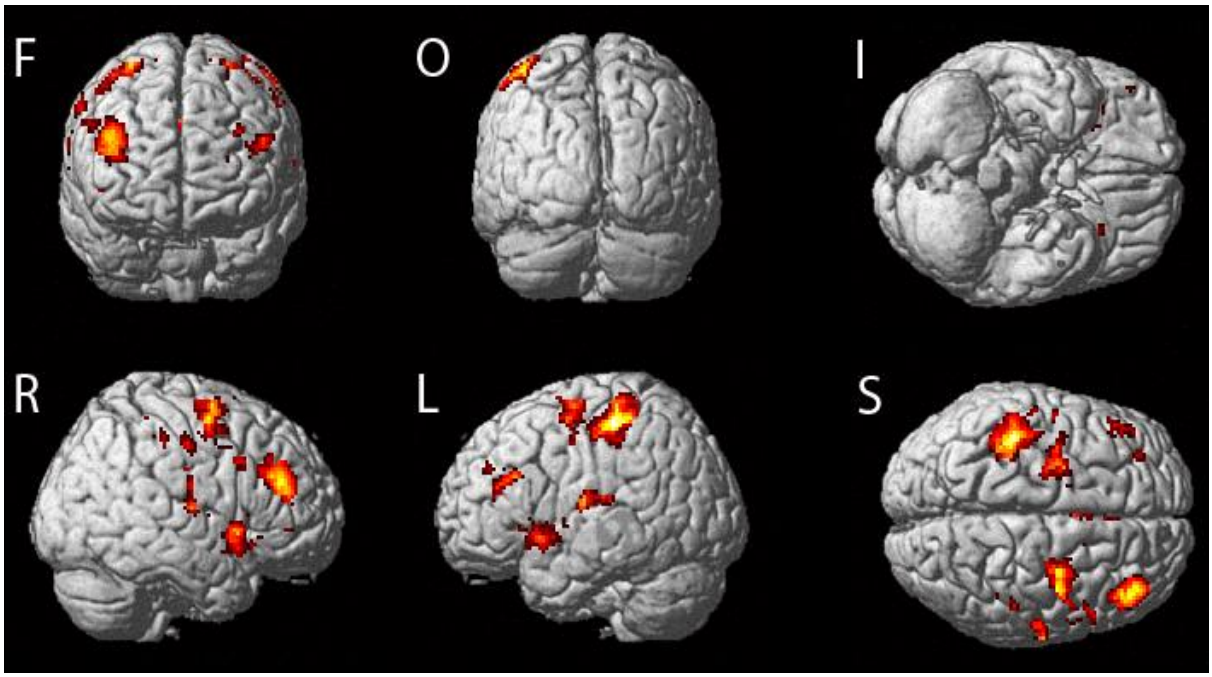
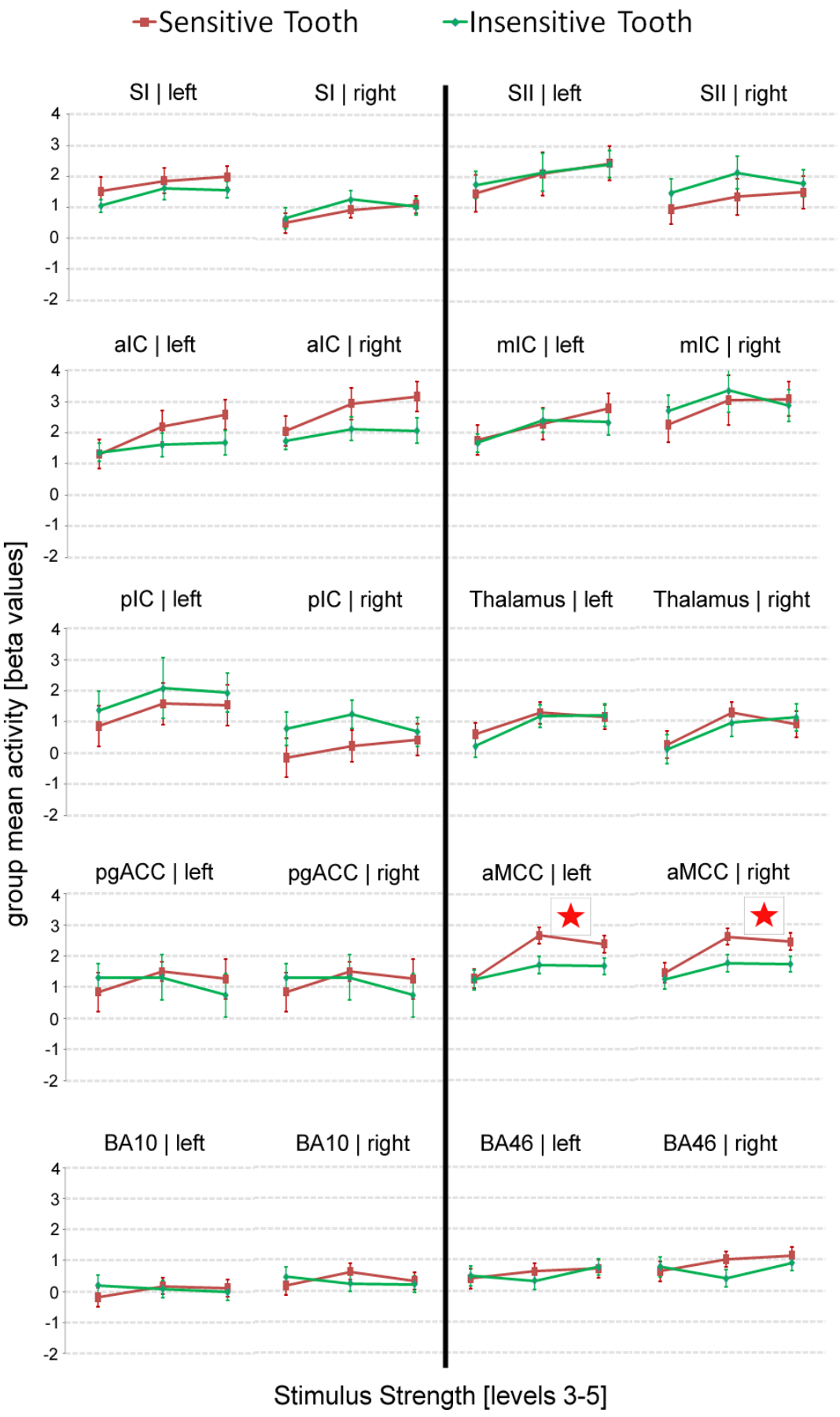


Figure 4: fMRI activation projected on the rendered MNI single subject T1 template. Illustrated is the brain activity in response to the pooled painful stimulus strengths of the sensitive tooth revealed via one sample t tests. A conservative statistical threshold (FWE-corrected with $p < 0.05$) has been chosen. F = frontal, O = occipital, R = right, L = left, I = inferior and S = superior.

## REGION OF INTEREST ANALYSIS

We found a significant main effect of tooth in the left aIC ($F = 6.41$, $p = 0.032$) as well as in the left and right aMCC ($F = 8.44$, $p = 0.017$ / $F = 12.46$, $p = 0.006$). No further significant main effects of tooth were observed in any other investigated region (Figure 5, Table 2).


Sensitive Tooth
Insensitive Tooth
SI | left
SI | right
SII | left
SII | right
aIC | left
aIC | right
mIC | left
mIC | right
pIC | left
pIC | right
Thalamus | left
Thalamus | right
pgACC | left
pgACC | right
aMCC | left
aMCC | right
BA10 | left
BA10 | right
BA46 | left
BA46 | right
group mean activity [beta values]
Stimulus Strength [levels 3-5]

5. Results of the ROI analysis. Illustrated is the relation between brain activity (y-axis, mean beta values) and the respective stimulus strengths (x-axis, levels 3-5). Red lines indicate the sensitive, green lines the insensitive tooth. Stars indicate a significant main effect of tooth. T-Bars indicate standard errors of the mean.

| **Brain Region (left \| right)** | **Main effect "Tooth"** | **p-value** | **F-value** |
|---|---|---|---|
| SI \| left | No | 0.35 | 0.96 |
| SI \| right | No | 0.31 | 1.14 |
| SII \| left | No | 0.75 | 0.10 |
| SII \| right | No | 0.40 | 0.65 |
| aIC \| left | Yes | 0.02 | 7.45 |
| aIC \| right | Yes | 0.02 | 7.92 |
| mIC \| left | No | 0.84 | 0.04 |
| mIC \| right | No | 0.83 | 0.05 |
| pIC \| left | No | 0.12 | 2.95 |
| pIC \| right | No | 0.18 | 2.08 |
| Thalamus \| left | No | 0.19 | 1.93 |
| Thalamus \| right | No | 0.26 | 1.45 |
| pgACC \| left | No | 0.98 | 0.00 |
| pgACC \| right | No | 0.37 | 0.87 |
| aMCC \| left | Yes | 0.02 | 8.84 |
| aMCC \| right | Yes | 0.01 | 9.33 |
| BA 10 \| left | No | 0.97 | 0.00 |
| BA 10 \| right | No | 0.27 | 1.40 |
| BA 46 \| left | No | 0.46 | 0.58 |
| BA 46 \| right | No | 0.20 | 1.96 |

Table 2. Results of the ROI analysis. Reported is the main effect "Tooth" of the repeated measures ANOVA with respective p- and F-values..

## DISCUSSION

The key finding of this study is that air blast stimuli of graded flow applied to sensitive and insensitive teeth evoked significant BOLD signal changes in several areas of the human brain. Compared to baseline neural activity, painful stimulations of sensitive teeth resulted in significant activations of somatosensory cortices SI and SII, insular and cingulate cortices, thalamus and frontal regions (Figure 4, Table 1). Activation of a similar modular network was previously reported in response to painful electric tooth stimulation (Ettlin et al., 2009, Weigelt et al., 2010, Brügger et al., 2011). Of particular interest was the head-to-head comparison between sensitive and insensitive teeth. Significant activation differences between sensitive and insensitive teeth for DH-pain vs. innocuous air stimuli were observed in anterior portions of the insular (aIC) and mid-cingulate cortex (aMCC) (Figure 5). These structures might therefore play specific roles in processing DH pain. Below, we discuss these two regions and their potential relation to DH pain in more detail.

### INSULAR CORTEX

Despite several interpretations and discussions about its functional specificity, the insular cortex is generally considered to play an important role within the nociceptive functional integration circuitry. Posterior portions seem more related to sensory aspects of pain while anterior parts are associated with emotional, cognitive and memory related aspects of pain perception (Apkarian et al., 2005). Craig et al. (2009) even postulate a posterior-to-mid-to-anterior pattern of integration of interoceptive sensory information in the insula. In a PET study they demonstrated a distinct stimulus processing pattern: objective sensory information processing in the posterior part was followed by integration of the information in the middle part and was finally re-represented more subjectively in the anterior part (Craig et al. 2000). In other words the incoming sensory stimulus receives it's subjective signature in the aIC., The aIC thus seems to be involved in the very subjective decision whether a stimulus is painful or not while posterior and middle portions of the insula most likely process and integrate objective information of incoming stimuli. This distinct posterior-to-anterior processing pattern is also depicted in our results. Objectively, the sensitive and the insensitive teeth received the same stimulus strengths which could be the reason for the non-significant differences between both teeth in the posterior portion of the insula. By contrast, the aIC seems to discriminate stimulus salience. From this perspective, it is

reasonable to assume that sensations from sensitive teeth inform the brain about a greater potential for damage. Hence activity differences within the aIC probably reflect the subjective interpretation whether the stimulation was painful or painless and whether a sensitive or insensitive tooth was stimulated, respectively.

### CINGULATE CORTEX

As in the aIC we observed a similar tooth specific activation pattern in the aMCC. Approximately 87% of pain imaging studies report activation of the anterior cingulate cortex (Apkarian et al., 2005). However, none of the cingulate cortex subdivisions is attributed a specific role for nociception. They are rather thought to serve as integrative processing domains related to several cognitive and emotional aspects of pain experiences. A recent expert report proposed a "cingulate premotor pain model" in which pain stimuli lead to autonomic and behavioral motor responses (Sikes et al., 2008). It has also been known from animal studies that cingulate cortex lesions produce a decrease in pain sensitivity and avoidance behaviour (Devinsky et al., 1995). Considering our results, the aMCC activity levels showed differential activity in response to stimulation of sensitive teeth (DH pain) and insensitive teeth (painless). This could be a consequence of higher arousal and stronger response to potentially harmful states since from a patient perspective, the painful air blasts may have had high negative valence. The stronger activation levels of sensitive teeth in the aMCC may indicate an initiation of avoidance behavior and motor preparation in response to DH pain.

### CONCLUSION

In the present feasibility study, we demonstrate that application of "natural" air stimuli to sensitive teeth induced cerebral activity patterns that share commonalities with the often described pain matrix that is formed by a modular organized brain network mainly activated by nociceptive inputs. Our neuroimaging data additionally provide evidence that differential activity in the aIC and aMCC may represent clinically relevant pain experienced by DH patients. Response patterns in these two brain regions may thus potentially serve as supplemental (objective) outcome measure for dental analgesic interventions in the future.

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## 2.3 STUDY III:

### EQUAL PAIN – UNEQUAL FEAR RESPONSE: ENHANCED SUSCEPTIBILITY OF TOOTH PAIN TO FEAR CONDITIONING

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ABSTRACT

Experimental fear conditioning in humans is widely used as a model to investigate the neural basis of fear learning and to unravel the pathogenesis of anxiety disorders. It has been observed that fear conditioning depends on stimulus salience and subject vulnerability to fear. It is further known that the prevalence of dental-related fear and phobia is exceedingly high in the population. Dental phobia is unique as no other body part is associated with a specific phobia. Therefore, we hypothesized that painful dental stimuli exhibit an enhanced susceptibility to fear conditioning when comparing to equal perceived stimuli applied to other body sites. Differential susceptibility to pain-related fear was investigated by analyzing responses to an unconditioned stimulus (UCS) applied to the right maxillary canine (UCS-c) versus the right tibia (UCS-t). For fear conditioning, UCS-c and USC-t consisted of painful electric stimuli, carefully matched at both application sites for equal intensity and quality perception. UCSs were paired to simple geometrical forms which served as conditioned stimuli (CS+). Unpaired CS+ were presented for eliciting and analyzing conditioned fear responses. Outcome parameter were 1) skin conductance changes and 2) time-dependent brain activity (BOLD responses) in fear-related brain regions such as the amygdala, anterior cingulate cortex, insula, thalamus, orbitofrontal cortex and medial prefrontal cortex. A preferential susceptibility of dental pain to fear conditioning was observed, reflected by heightened skin conductance responses and enhanced time-dependent brain activity (BOLD responses) in the fear network. For the first time, this study demonstrates fear-related neurobiological mechanisms that point towards a superior conditionability of tooth pain. Beside traumatic dental experiences our results offer novel evidence that might explain the high prevalence of dental-related fears in the population.

## INTRODUCTION

Experimental fear conditioning has proven to be a valuable tool for studying the neurobiological underpinnings of (pain-related) fear, anxiety, specific phobias and placebo analgesia (Bradley et al., 2008; Cheng et al., 2003; De Peuter et al., 2011; Delgado et al., 2006; Dunsmoor et al., 2013; Lui et al., 2010; Phelps et al., 2004; Schiller et al., 2008; Schweckendiek et al., 2011). Fear conditioning entails a learning process in which a predictive association is acquired between a previously neutral stimulus (i.e. the conditioned stimulus, CS) and a fear-evoking stimulus (i.e. the unconditioned stimulus, UCS). Following a number of paired presentations of the CS and UCS, the sole presentation of the conditioned stimulus (CS+) is sufficient to elicit an emotional response (conditioned response, CR) similar to that evoked by the UCS.

Regarding the neural basis of fear conditioning, studies point to the amygdala as a key structure of fear learning (Buchel et al., 1998; LaBar et al., 1998; Phelps et al., 2004). But such findings are not consistent. Some studies failed to detect amygdala responses during fear conditioning (Fischer et al., 2000; Fischer et al., 2002; Jensen et al., 2003; Knight et al., 1999; Knight et al., 2004). Importantly, a constellation of other structures such as the orbital frontal cortex (OFC), the thalamus, anterior cingulate cortex (ACC), the insula and the medial prefrontal cortex (mPFC) are linked to aspects of fear conditioning (for a current review, see Sehlmeyer et al.,2009). These structures modulate fear responses and extend them to the wider context of the conditioning (Fiddick, 2011).

It has been observed that fear conditioning depends on stimulus salience. Interestingly, some classes of stimuli appear to be more readily associated with the UCS, leading to more pronounced CR development and greater resistance to CR extinction. This has been observed for biologically salient stimuli like spiders and angry faces (Ohman and Dimberg, 1978; Ohman and Soares, 1993; Schweckendiek et al., 2011). In support of this observation, Seligman (1971) found that human fears and phobias are not randomly distributed in the population, thus suggesting the presence of specific underlying mechanisms for fear development. Dental phobia is of particular interest in this regard as it is one of the most prevalent phobias and should be considered as a specific phobia (van Houtem et al., 2013). It is a remarkably severe condition with protracted duration and resistancy to treatment (Agras

et al., 1969; Fiset et al., 1989; Oosterink et al., 2009; Ost, 1989; 1997). Dental phobia is defined as the excessive and uncontrollable fear of dental treatment, whereas the majority of phobics indicate that fear of pain and feelings of helplessness are the main reasons for their intense dental anxiety (Scharmuller et al., 2014). Furthermore, dental phobia is unique as no other body part is associated with a specific phobia.

It follows from the pertinent literature and the foregoing considerations that dental pain might exhibit enhanced fear responses compared with other bodily pains. Working on this basis that tooth pain is more susceptible to fear conditioning, we expected to find a stronger CR of dental stimuli (CS+c) compared with tibial stimuli (CS+t), the latter serving as a control. After equalizing the UCS pain intensity and quality at both stimulation sites (UCS-c, UCS-t, respectively), we expected differential CRs by analyzing skin conductance responses (SCR) and brain activity (blood oxygenation level dependent, BOLD) in fear-related brain regions (ACC, amygdala, insula, thalamus, OFC and mPFC).

## MATERIAL AND METHODS

### SUBJECTS

On the basis of a stringent selection process, twenty-one healthy subjects (mean age 32.3, SD ±8.2, 12 females) reporting regular visits to dentists (and/or dental hygienists) participated in the study. Exclusion criteria included systemic disease, caries, large restorations, periodontal disease, dental anxiety/phobia or a history of trauma or sensitivity of maxillary canines. Four subjects did not fulfill the criteria of the pain matching procedure (see below for criteria), three subjects were excluded from the SCR analysis due to technical failure of the recording system, and two subjects were excluded because they did not develop contingency awareness. These exclusions resulted in a total sample of n = 15 for fMRI analysis and n =12 SCR datasets. The study and all procedures and consent forms were approved by the local Ethics Committee. Subjects received 50 Swiss Francs per hour for participation.

### INTERVIEW AND ANXIETY SCALES

In order to compare the relevance of both stimulation sites for fear, subjects were carefully selected to ensure no history whatsoever of dental or tibial-related anxiety. In an interview

session preceding the conditioning experiment and without giving any indication as to the reason for the interview, subjects were required to report experience in any form of a traumatic event at the dentist or dental hygienist or of any injuries to the dentition or tibial region. Potential subjects were excluded from participation if they reported any traumatic event or injury. To exclude possible anxiety-mediated effects associated with dental stimulation, participants completed the Dental Anxiety Scale (DAS), which is one of the most often used dental fear instruments (Corah, 1969). DAS scores below 13 points indicate mild to no dental anxiety. Subjects scoring in excess of 13 points were excluded from further participation. Given the relationship between dental anxiety and general fears and anxiety (Fuentes et al., 2009), we applied also the State-Trait Anxiety Inventory (STAI), the most widely used self-report measure of anxiety (Spielberger et al., 1983). The STAI state is suitable as a screening instrument for predicting anxiety disorders (Kvaal et al., 2005). A cut-off point of 39-40 indicates clinically significant symptoms of state anxiety (Knight et al., 1983). Subjects with a score above 39 points were excluded.

### ELECTRIC STIMULUS DELIVERY

A modified “Compex Motion” system (Compex Médical SA, Ecublens, Switzerland) was used as described by Keller et al. (2002). This stimulation has been proven to evoke reliable sharp and pricking pain sensations (Brugger et al., 2011; Brugger et al., 2012; Keller et al., 2002). The Presentation® software (http://www.neurobs.com/presentation) was used to control the experimental protocol. Shielded wires were used to avoid radiofrequency contamination by the stimulation current.

### TIBIAL STIMULUS APPLICATION

Small hydrogel surface electrodes (28x20mm, Ambu A/S, Denmark) were used for tibial stimulations (Figure 1). The electrodes were placed on the anterior border of the tibia at a distance of 1cm. Care was taken that the tibialis anterior muscle was unaffected by the stimulation.

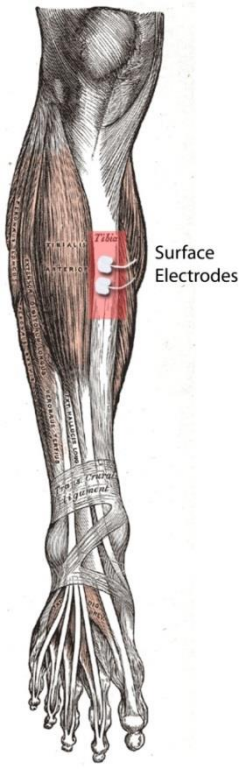


Figure 1. UCS delivery site (right tibia). It shows the placement of the electrodes on the anterior border of the tibia.

## DENTAL STIMULUS APPLICATION

Blu-Mousse (Thixotropic Vinyl Polysiloxane, Edgewood, MD, USA) impressions were taken from the subject's dentition (Gutzeit et al., 2011; Meier et al., 2012). Stainless steel electrodes were embedded in each splint at the labial and palatal centers of the right upper canine (Figure 2). To minimize electric resistance, we placed a 3-mm round piece of hydrogel (Klusapotheke, Zurich, Switzerland) on the electrodes. Care was taken that the splint itself did not evoke pain or discomfort.

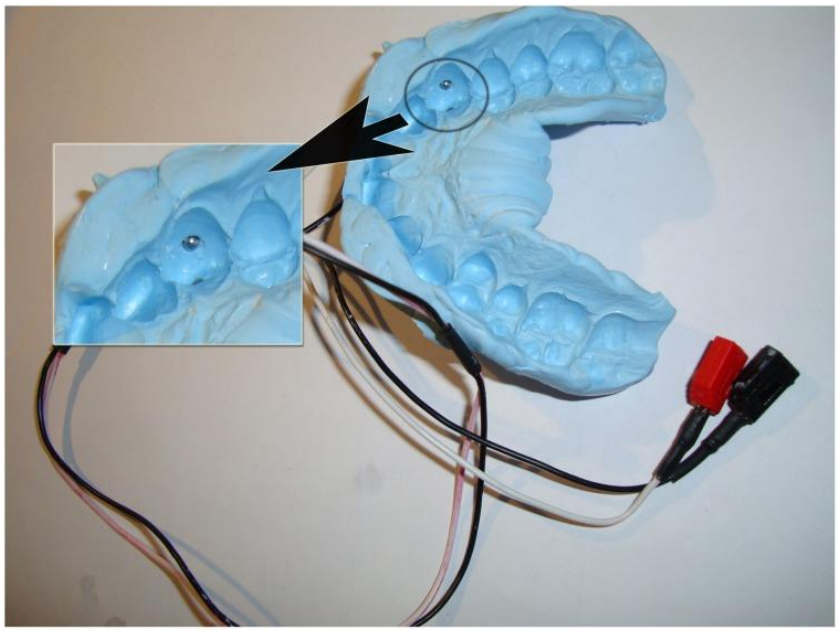

Figure 2. UCS delivery site (right canine). This figure illustrates an individual dental splint with embedded electrodes.

## MATCHING OF UCS PAIN INTENSITY AND QUALITY

Fiber specificity plays an important role in experimental pain. A-delta and C fibers are major pain-conducting nerve fibers and are thought to activate different cortical regions within the "pain matrix" (Matre et al., 2010). A-delta fibers evoke an initial sharp, pricking and well-localized pain experience, whereas C fibers elicit dull and prolonged perceptions (Bishop et al., 1958). We aimed to evoke a pricking pain experience at both stimulation sites, thus activating mainly A-delta pain fibers in the following three-step procedure.

Firstly, we applied different intensities of electric current in ascending order and asked subjects to report their respective pain experience as either "pricking", "dull" or "pressing". These three verbal descriptors best permit discrimination between A-delta and C-fiber mediated pain experience with a specificity and sensitivity over 95% (Beissner et al., 2010). Potential subjects who did not report the perception of pain to be "pricking" were excluded from the study.

Second, we applied different intensities of electric current according to an adaptive staircase method (Figure 3). This method entails the presentation of a sequence of stimuli, each of which is judged after presentation concerning perceived intensity. The stimulus strength is adjusted to progressively increase or decrease until the judged intensity changes. Upon

change, the stimulus intensity is reversed. This technique is widely accepted as robust in the detection of pain thresholds and shows reduced between-session variability and improved reliability compared with other methods (Cornsweet, 1962; Yarnitsky and Sprecher, 1994). In the MR scanner but preceding the conditioning paradigm, subjects were asked to rate the perceived intensity of pain on a visual analog scale (VAS), with the endpoints “0” (no pain) and “10” (worst imaginable pain). Alternating the stimulation site, we applied pulses of electric current in steps of 1mA with an inter-trial interval randomized between 8 and 12 s. Whenever the rating on a stimulation site exceeded or fell below the hypothetical threshold of “5” (i.e. the transition point corresponding to a painful but tolerable experience), the stimulation algorithm randomly chose for the following stimulation of that particular stimulation site one of the three possible next higher intensities. If, for the following stimulation, the subject rated again a “5” or higher, the stimulus intensity was reversed until the subject rated below a “5”. After this, a random stimulus intensity from one of the three next-lower intensities was applied. If the subject then rated below a “5”, the algorithm reversed again and intensities were increased until the subject rated a “5” or higher. This procedure was performed until stimulation at both stimulation sites reached the transition point four times in succession after alternating between the stimulus sites.

Finally, the intensity of the electric shock was taken as the mean value of the four transition points, serving as the individual UCS for each stimulation site. Potential subjects who did not reach the transition point of “5” within each of the four runs were excluded. To guarantee stable perceptions of stimulus intensity, the whole pain matching procedure was repeated after the extinction phase. To allow for parametric testing of the UCS ratings, we performed a Kolmogorov-Smirnov test which tested for normality of the data. To further control for differences in perceived pain quality, post-experiment valence ratings (unpleasant/pleasant) were collected by using a five-point self-assessment manikin (SAM) scale. To assess possible differences in mean ranks, the non-paramteric Wilcoxon signed-rank test was used. Furthermore, subjects who reported a difference in UCS valence of more than one point were excluded.

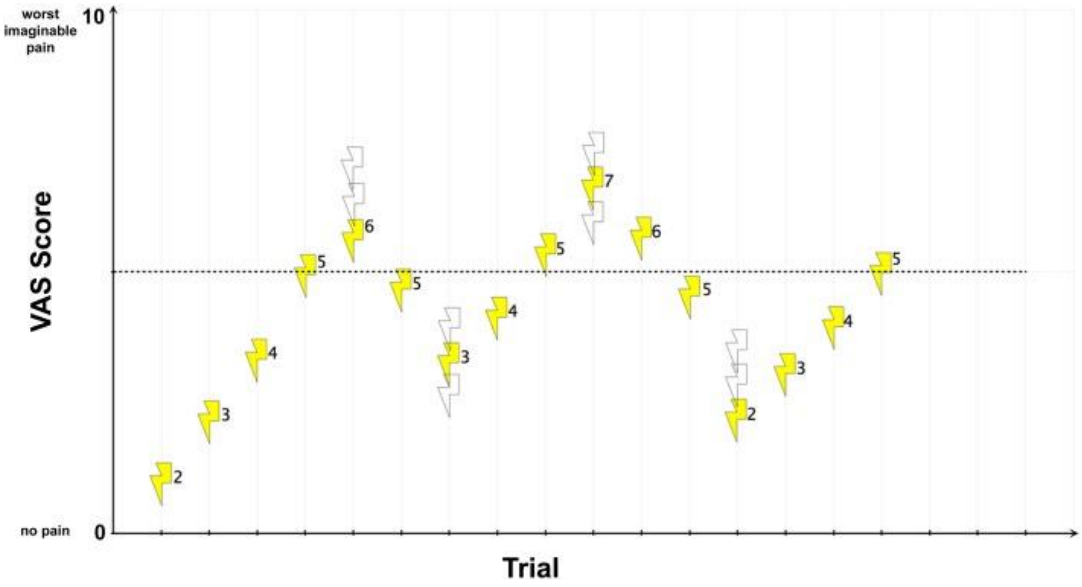


Figure 3. UCS pain intensity and quality matching. For illustration, exemplary electric current strengths (mA-values) are shown next to each stimulus. In this example, an electric current strength of 5 mA reached the transition point. Open symbols represent other possible stimulus intensities that might have been chosen by the randomization procedure.

## FEAR CONDITIONING PROCEDURE

The experiment consisted of an acquisition phase followed by an extinction phase (30 unreinforced trials, 10 per CS). Only data of the acquisition phase is reported in the present study. During the acquisition phase a 50% partial reinforcement conditioning strategy was applied which allowed for a UCS-free comparison of both CS. This approach was successfully used in other fear conditioning experiments and permits the analysis of fear responses without confounding effects of the UCS (Buchel et al., 1998; Dunsmoor et al., 2007; Moessnang et al., 2013). The three CS consisted of simple geometrical forms: a triangle, a circle and a square. These were presented in a pseudo-randomized order (no more than two consecutive trials) and in white color on a black background (CS duration 2 s, inter-trial interval 8-12 s). Assignment of the geometrical form to the different US was randomized across subjects. One CS (CS-) was never paired with an electric shock. The UCS, having a duration of 1ms, co-terminated with the CS presentation. During the acquisition phase, a total of 150 visual stimuli were presented. These consisted of 30 CS-, 30 unconditioned and conditioned stimuli of each type (UCS-c, UCS-t and CS+c, CS+t, respectively). Subjects were instructed that each of the geometrical forms could be followed by an electric shock, either to the canine tooth or to the shinbone.

## CONTINGENCY AWARENESS

Although still debated, controlling for contingency awareness is important in order to reduce differences in the dependent variables (Hamm and Weike, 2005; Lovibond and Shanks, 2002; Tabbert et al., 2011). Subject awareness of the reinforcement contingencies was assessed immediately after the extinction phase in an interview conducted in the control room outside the magnet. Subjects were asked to choose which type of geometric figure preceded the different UCS types using a forced choice questionnaire.

## SKIN CONDUCTANCE RESPONSES

SCR were acquired using the constant voltage (0.5 V) method by means of MRI-compatible and radiotranslucent electrodes with a 1 cm diameter contact area placed on the distal phalanges of the second and third finger of the participant's left hand (BIOPAC Systems Inc., Goleta, CA). The SCR signal was amplified and recorded with a BIOPAC Systems skin conductance module connected to an Apple MacBook Pro running AcqKnowledge software version 4.0 (BIOPAC Systems Inc., Goleta, CA). Data were recorded with a sampling rate of 200 Hz. The RF-artifacts in the SCR-waveforms were removed off-line by a median-filter (window length: 50 samples) using the software MATLAB R2011b (MathWorks, Natick, MA). Off-line analysis of SCR waveforms was done using the automated scoring system for EDA data included in the AcqKnowledge software. The window length was set to 6s, starting at the CS presentation. Only SCRs were analyzed with response amplitude higher than 10% of the maximal response. The SCRs were then normalized through a square root transformation. Statistical analyses were performed using paired t-tests as implemented in the software PASW Statistics (Version 18, SPSS Inc.). To be consistent with the fMRI analysis (see below), we divided the acquisition phase in an early (3rd to 16th trial) and late phase (17th to 30th trial).

## FMRI PROTOCOL

Functional and anatomical scans were obtained using a 3-T Phillips Achieva scanner with an 8-channel receive-only head coil. We used a blood-oxygen-level dependent (BOLD) sensitive single-shot gradient echo-planar imaging sequence to acquire 33 axial whole brain slices. Parameters were as follows: echo time = 30 msec, flip angle = 75 degrees, repetition time =

2526 ms, slice thickness = 4 mm, inter-slice gap = 0 mm, field of view = 220 mm, and matrix size in plane = 128 x 128, resulting in a voxel size of 1.72 x 1.72 x 4 $mm^3$. Three dummy scans were first acquired to reach steady-state magnetization and subsequently discarded. 180 high-resolution T1- weighted axial slices (spoiled gradient echo) were acquired with TR = 20 ms, flip angle = 20°, voxel size = 0.98 x 0.98 x 1.02mm3, FOV = 24 cm, and matrix = 256 x 192; these were used as an underlay for individual functional maps., The acquisition phase of 930 functional images lasted about 28 min and was followed by an extinction phase of approx. 10min.

SPM8 (http://www.fil.ion.ucl.ac.uk/spm) software package running on MATLAB R2011b (Mathworks, Natiek, USA) was used for functional voxel-by-voxel analysis. After slice timing, spatial realignment to the first image in the series as reference was performed and it was assured that detected movement did not exceed 2 mm (translational) or 1° (rotational) in relation to the reference. For studying group effects, data were normalized to the MNI template brain (Evans et al., 1992) followed by smoothing with a Gaussian kernel of 8 mm full-width-at-half-maximum (FWHM). To control for possible head movement effects, individual movement parameters (translations in x, y and z-direction, as well as rotations around x, y, and z axis) were implemented in the $1^{st}$ level model as regressors of no interest. Individual SCR amplitudes (N = 12) were included as additional regressors of no interest to account for possible differences in brain activity explained by differential SCR levels. The first two trials of each CS were discarded from analysis because learning could not have occurred yet (Merz et al., 2013; Phelps et al., 2004; Schweckendiek et al., 2011). The high-pass filter was set to 128s and the regressors were convolved with the canonical hemodynamic response function implemented in SPM8. To account for gradual development of fear expression, we divided the acquisition phase in an early (3rd to 16th trial) and late phase (17th to 30th trial) (LaBar et al., 1998; Schiller et al., 2008; Tabbert et al., 2005). For each subject, the following experimental conditions were modeled: CS+c, CS+t, CS- (early and late phase each), UCS-c and UCS-t. The CS regressor onsets were set to coincide with the presentation of the CS with a duration of 2 seconds. The UCS onsets were set 2 seconds after CS presentation. Statistical parametric maps were then calculated, yielding beta estimates of the model fit for each subject and condition. The random effects group analysis was performed by using one-sample t-tests. The contrasts CS+c>CS-, CS+t>CS-, CS+c>CS+t and

CS+t>CS+c were computed for the early and late phase of the acquisition. Resulting voxel T-values were color-coded and superimposed onto the MNI single-subject-T1 brain using MRcroGL (http://www.cabiatl.com/mricrogl/). For visualization purposes, we used a whole-brain statistical threshold of $p < 0.001$ (uncorrected) with a voxel extend threshold of 10 voxels.

In a subsequent region-of-interest (ROI) analysis, we investigated the following bilateral brain structures: amygdala, insula, ACC, OFC, thalamus and the mPFC. The ROI masks were taken from the probabilistic Harvard-Oxford Cortical and Subcortical Structural Atlas (http://www.fmrib.ox.ac.uk/fsl). The probability threshold for belonging to the respective brain region was set to $p > .25$. To further assess the success of the UCS matching procedure, we additionally introduced the posterior part of the insula as a control region which was parcellated after Brooks (Brooks et al., 2002; Brooks et al., 2005). This part of the insula is related to sensory aspects of pain (Craig, 2009; Garcia-Larrea, 2012) and appears to be the only part of the cerebral cortex where intra-cortical electric stimulation is able to trigger experience of somatic pain (Mazzola et al., 2012). The failure to find significant differences between UCS-c and UCS-t in this region would provide additional support for the equivalence of subjective pain intensities. All ROI analyses were computed using the small volume correction implemented in SPM8. Only clusters which survived a familywise error rate (FWE) correction were reported.

## RESULTS

### ANXIETY SCALES AND INTERVIEW

None of the subjects recalled any traumatic event at the dentist or dental hygienist or any traumatic injuries in the tibia region. All subjects showed scores for state and dental anxiety in a low, non-clinical range, with a mean DAS score of 7.46 (SD ±1.50) and a mean STAI score of 29.35 (SD ±4.51).

### PAIN INTENSITY AND QUALITY MATCHING

None of the participants reported any painful or uncomfortable sensations associated with the dental splint or tibial electrodes themselves. 4 of 21 participants did not reach the transition point of “5” on the VAS scale and were excluded. The Kolmogorov-Smirnov test

indicated normality of the UCS rating data (Z = 0.90, p = 0.39). Pre- and post-experiment pain matching revealed that slightly higher currents were needed for the canine tooth to reach the transition point compared with tibial stimulations (pre-experiment mA-values [Mean± SEM]; Tooth: 17.04 ± 1.42, Tibia: 14.90 ± 1.49 / post-experiment mean mA-values [Mean ± SEM]; Tooth: 17.32 ± 1.17, Tibia 15.77 ± 1.52) (Figure 4). However, these differences were not significant (pre-experiment T= 0.74; p = .48 / post-experimental T = 0.43; p = .68). Furthermore, pre- and post-experiment differences within UCS-c and UCS-t intensities were not significant (UCS-c: T = -0.16; p = .88 / UCS-t: T = -0.40; p = .70). To control for sensitization or habituation effects, or any other changes in perception of the electric stimulus, we calculated the intraclass correlation coefficient (ICC) between individual pre- and post-experiment electric current strengths required during the pain matching procedure to reach the transition point. We observed an ICC of 0.788 (F = 8.419, p = .001) for UCS-t- and 0.745 (F = 6.857, p = .003) for UCS-c-intensities, pointing towards highly stable thresholds. Regarding pain quality, all participants reported a short and pricking pain perception. Furthermore, post-experimental SAM ratings did not show any significant differences between UCS-c and UCS-t, as revealed by Wilcoxons test (Z = -1.385; p = .166).

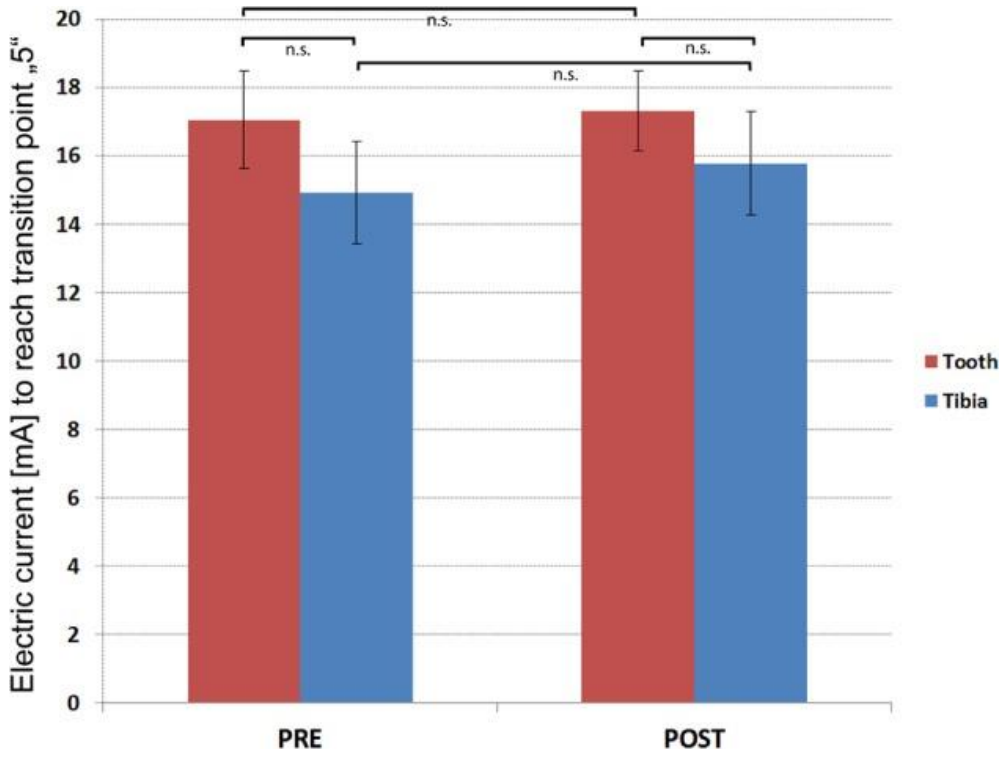


Figure 4. Results of the pain matching. Y-axis illustrates the group (*N* = 15) mean electric current (mA) that was needed to reach the transition point. T-bars indicate standard errors of the mean (±s.e.m.).

## SKIN CONDUCTANCE RESPONSES

### Early phase of acquisition

Paired t-tests of the autonomic responses of CS+c revealed significantly stronger SCR compared to CS+t (T = 2.28, p = .02), although both stimuli were rated as equally painful (Figure 6). No significant differences could be found between CS+c and CS- and CS+t and CS-.

### Late phase of acquisition

As in the early phase, paired t-tests of CS+c showed significantly stronger SCR than the CS+t (T = 2.39, p = .02) (Figure 6). Again, no significant differences could be found between both CS+ and CS-.

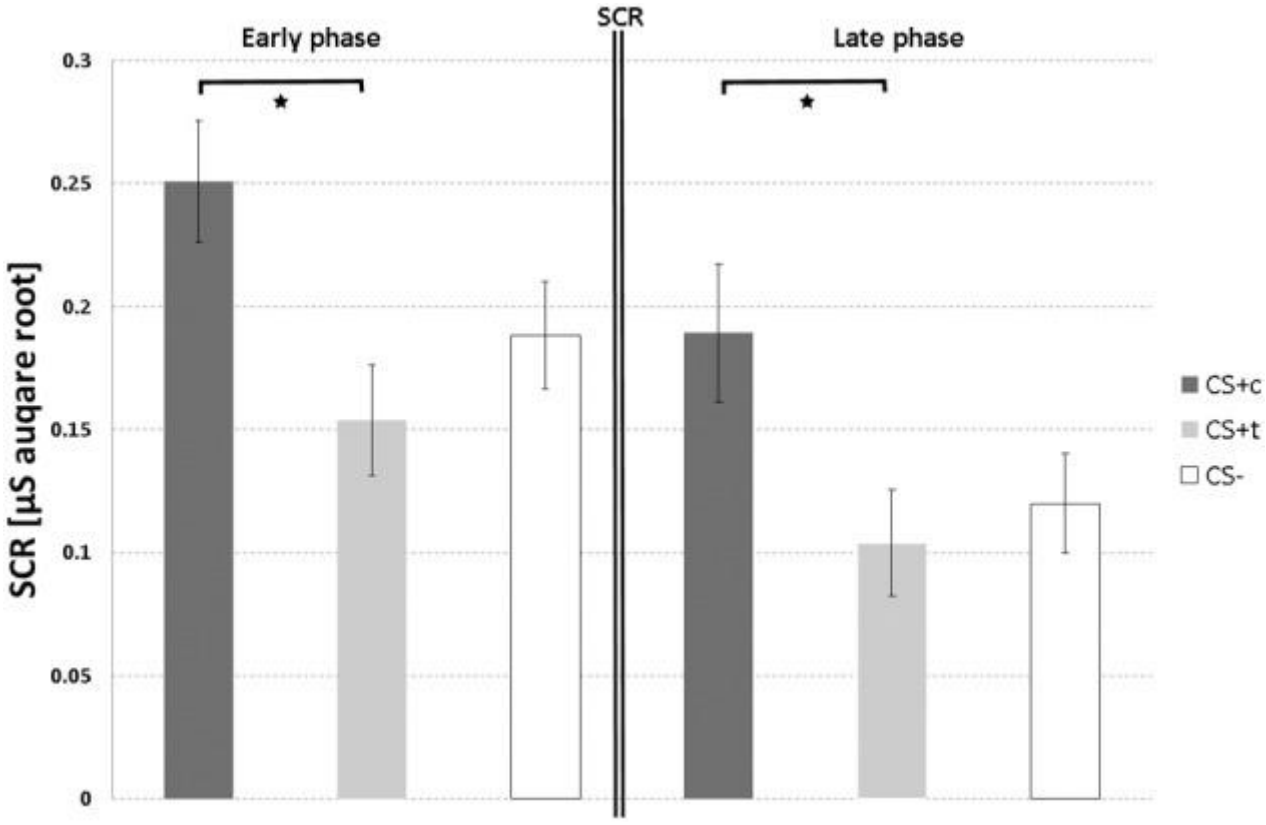


Figure 5. SCR responses (µS, square root transformed, *N* = 12) over both acquisition phases. T-bars represent standard errors of the mean (±s.e.m.). $^{*}p < 0.05$.

## FMRI RESULTS

### Unconditioned responses

Figure 5 shows the comparison between UCS-c and UCS-t in the posterior insula ([Mean estimates ±SEM]; UCS-c: 0.93 ± 0.21. UCS-t: 0.73 ± 0.15). The paired t-tests revealed no

significant results (T = 0.68, p = .51). However, both UCS showed significantly higher activation compared to the non-UCS (UCS-c vs non-UCS: T = 2.95, p = .01; UCS-t vs non-UCS: T = 3.65, p = .01).

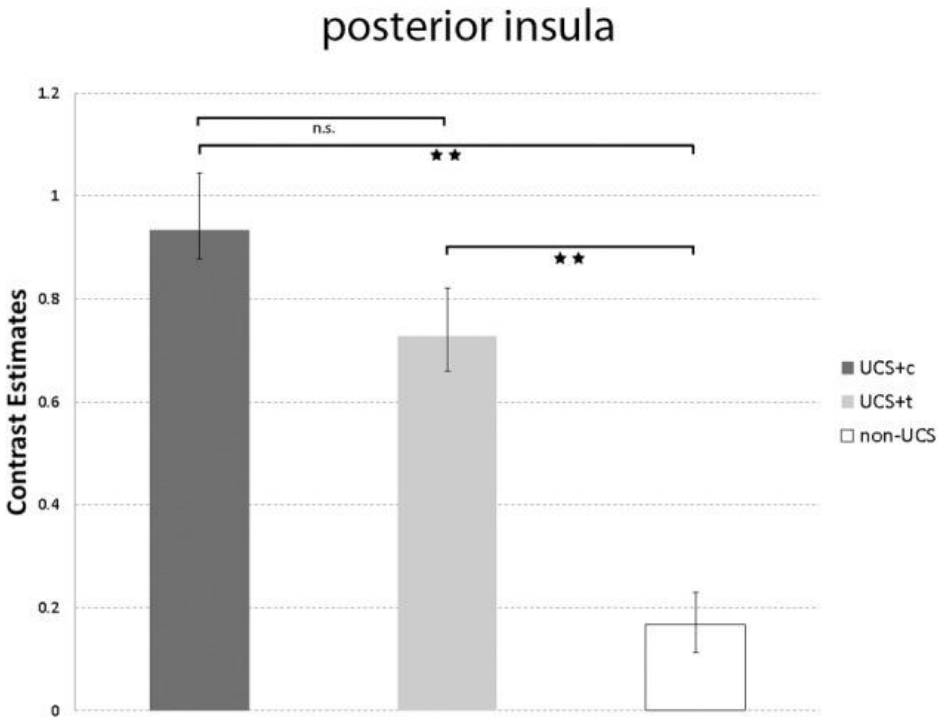


Figure 6. Illustrated are the mean contrast estimates of the unconditioned responses UCS-c, UCS-t, and Non-UCS in the posterior insula. T-bars represent standard errors of the mean (±s.e.m.). $^{**}p < 0.01$.

## Conditioned responses

For the early and late phase of acquisition, peak coordinates, t-values and corrected p-values of the respective contrasts are shown in Table 1.

### Early phase of acquisition

The whole-brain analysis of the contrast CS+c > CS- revealed a single cluster in the right OFC (Peak MNI 34 22 -20, T = 5.45, p < 0.05, FWE-corrected). The respective ROI analysis (based on small-volume correction) revealed significantly higher responses in the bilateral anterior midcingulate cortex (aMCC), the right amygdala, bilaterally in the anterior insula, the OFC and thalamus (p < 0.05, FWE-corrected). Regarding the contrast CS+t > CS- no significant activations could be found. The comparison CS+c > CS+t revealed a significant cluster in the aMCC in the whole-brain-analysis (Peak MNI -4 28 32, T = 5.66, p < 0.05, FWE-corrected).

Further ROI analysis yielded significant activations bilaterally in the anterior insula, OFC and Thalamus (Figure 7). The reverse contrast CS+t > CS+c did not show any significant results.

Late phase of acquisition

The contrast CS+c > CS- revealed no significant results. Similary, the comparisons CS+t > CS- and CS+c > CS+ t did not show any significant results. However, the contrast CS+t > CS+c showed significantly higher responses in the mPFC ROI ($p < 0.05$, FWE-corrected, Figure 8).

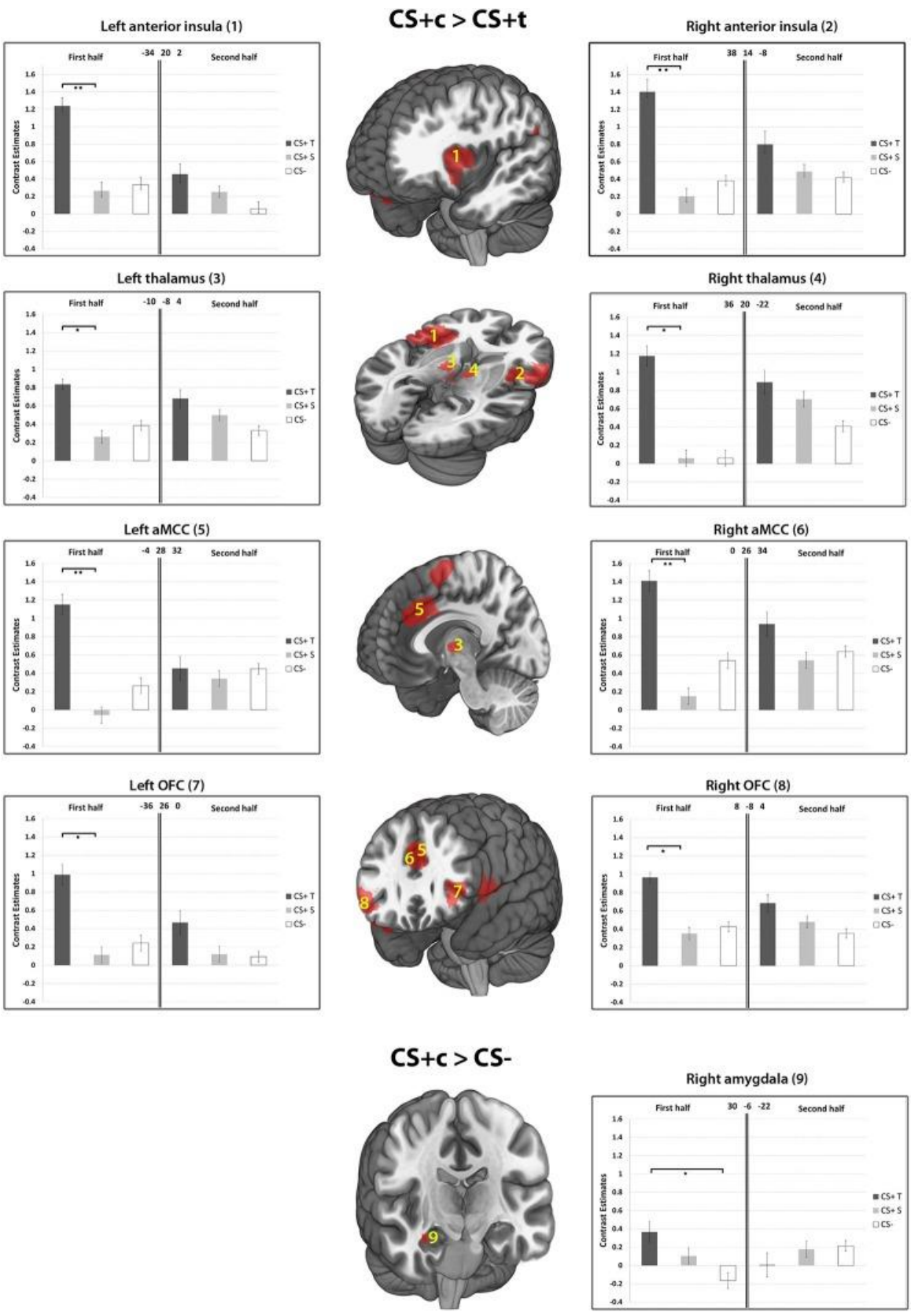


Figure 7. Results of the contrasts CS+c > CS+t, and CS+c > CS− within ROIs 1–9. Whole-brain SPM activations maps are shown with a statistical threshold of $p < 0.001$, uncorrected, voxel threshold = 10. Mean contrast

estimates (and standard errors of the mean ± s.e.m.) for early and late phases in the respective peak voxels are illustrated in the bar graph. $^{*}p < 0.05$; $^{**}p < 0.01$.

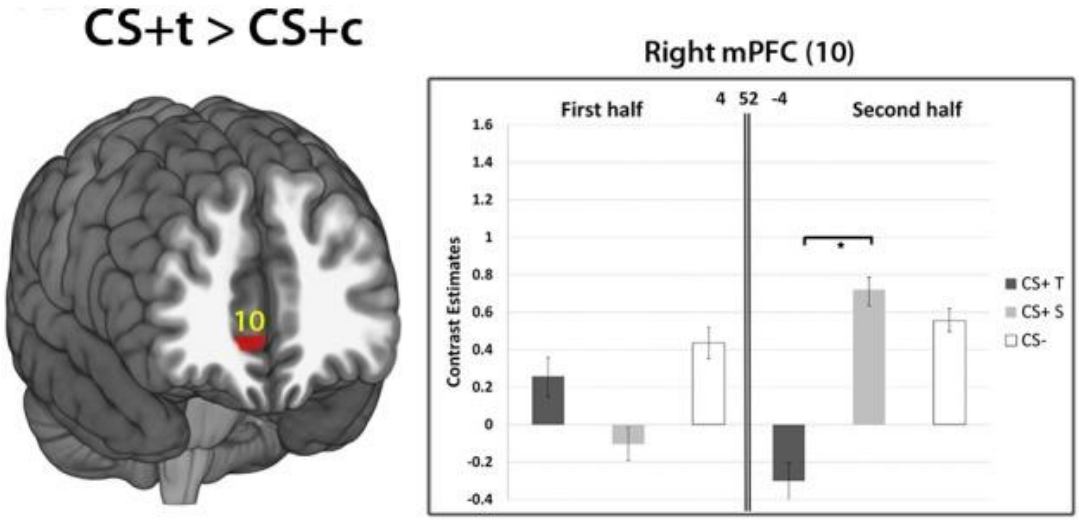


Figure 8. Results of the contrast CS+t> CS+c within the mPFC ROI (10). Whole-brain SPM activations maps are shown with a statistical threshold of $p < 0.001$, uncorrected, voxel threshold = 10. Mean contrast estimates (and standard errors of the mean ± s.e.m.) for early and late phases in the respective peak voxels are illustrated in the bar graph. $^{*}p < 0.05$.

| **Acquisition phase** | **Contrast** | **Brain region** | **$T_{max}$** | **$p_{corr}$** | **x** | **y** | **z** |
|---|---|---|---|---|---|---|---|
| Early phase | CS+c > CS- | Left aMCC | 4.31 | 0.014 | -2 | 22 | 36 |
| | | Right aMCC | 4.30 | 0.014 | 6 | 32 | 26 |
| | | Right amygdala | 3.80 | 0.022 | 30 | -6 | -22 |
| | | Left anterior insula | 4.78 | 0.026 | -30 | 22 | 2 |
| | | Right anterior insula | 5.40 | 0.001 | 36 | 16 | -14 |
| | | Left OFC | 5.13 | 0.001 | -30 | 24 | -8 |
| | | Right OFC | 5.45 | 0.001 | 34 | 22 | -20 |
| | | Left thalamus | 3.59 | 0.038 | -12 | -12 | 0 |
| | CS+t > CS- | No significant results | | | | | |
| | CS+c > CS+t | Left aMCC | 5.66 | 0.001 | -4 | 28 | 32 |
| | | Right aMCC | 5.51 | 0.001 | 0 | 26 | 34 |
| | | Left anterior insula | 4.96 | 0.001 | -34 | 20 | 2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Right anterior insula | 5.19 | 0.001 | 38 | 14 | -8 |
| | | Left OFC | 4.63 | 0.006 | -36 | 26 | 0 |
| | | Right OFC | 4.82 | 0.003 | 36 | 20 | -22 |
| | | Left thalamus | 4.34 | 0.011 | -10 | -8 | 4 |
| | | Right thalamus | 4.13 | 0.022 | 8 | -8 | 4 |
| | CS+t > CS+c | No significant result | | | | | |
| Late phase | CS+c > CS- | No significant results | | | | | |
| | CS+t > CS- | No significant results | | | | | |
| | CS+c > CS+t | No significant results | | | | | |
| | CS+t > CS+c | Right mPFC | 3.41 | 0.042 | 4 | 52 | -4 |

Table 1. Results of the conditioned responses in early and late phases of the acquisition phase. Peak voxels and p-values are shown for the contrasts CS+c > CS-, CS+t > CS-, CS+c > CS+t and CS+t > CS+c. The threshold for the ROI analysis (small volume correction) was set to $p < 0.05$ (FWE-corrected according to SPM8). Coordinates are reported in the MNI space.

## DISCUSSION

In the current study, we asked the question whether painful stimuli applied at the tooth and tibia evoke different fear responses while having subjectively identical intensity and quality. The finding of such selectivity in fear responses of healthy subjects would lend weight to the idea that the underlying brain mechanisms responsive to the two different sites are not quite the same and that this difference is potentially associated and thus contributes in some way to the development of specific phobias such as dental phobia. In order to directly compare brain activity and SCR between anticipated dental and tibial shocks, it was crucial to match the UCS at both stimulation sites in subjectively perceived pain intensity and quality. The success of our UCS matching procedure is not only depicted in pre- and post-experiment measurements, but also in non-significant differences between UCS-c and UCS-t responses in the posterior insula. This part of the insula has been proposed as a potential "primary cortex for pain" (Garcia-Larrea, 2012) and constitutes a promising biomarker for pain (Wager et al., 2013).

As hypothesized, our results provide strong evidence in favor of heightened susceptibility of CS+c to fear conditioning in subjects without a history of dental fear. This evidence is

provided on the basis of two independent but concurrently applied methods, namely SCR and BOLD responses. As a main finding, enhanced brain activation of CS+c compared to CS+t could be found in regions of the fear network including the aMCC, the anterior insula, the OFC and the thalamus. These activations were exclusively present in the early phase of acquisition, which is in line with other studies reporting fear related brain activation in the first half of the acquisition phase (Schiller et al., 2008; Schweckendiek et al., 2011). Enhanced responses of the amygdala could only be found in the comparison CS+c > CS-. Several lines of evidence point towards the amygdala as a key neural system underpinning fear learning and extinction (Buchel et al., 1998; LaBar et al., 1998; leDoux, 1996). However, a recent review of 44 fear conditioning studies showed that 19 of these failed to find amygdala activation (Sehlmeyer et al., 2009). Previous results from fear conditioning studies indicate that the amygdala is involved during the initial learning phase only, showing rapid habituation after a few trials (Bach et al., 2011; Buchel et al., 1998; LaBar et al., 1998; Marschner et al., 2008). The OFC has also been implicated in aspects of fear learning and has been labeled the "extended amygdala", together with other structures such as the bed nucleus of the stria terminalis The finding of enhanced amygdala and OFC activity solely in the first half of the CS+c > CS- condition supports our hypothesis regarding enhanced susceptibility of of CS+c to fear conditioning.

There have long been doubts about the adequacy of animal fear conditioning models (which favor the amygdala as a core structure) in explaining anxiety disorders (Fiddick, 2011). Recently, this traditional view of the amygdala has been extended by an involvement of several other brain regions which play an important role in fear learning and expression. In maintaining extensive inputs from the amygdala (Vogt, 2005), the ACC is involved in the anticipation of threat, aware conditioning, response selection and in the interpretation of interoceptive states (Mechias et al., 2010; Merz et al., 2013; Paulus and Stein, 2006). These interoceptive states are integrated in the anterior insula (Craig, 2002) and are often associated with intensive aspects of affective components which can provoke strong withdrawal actions. It has been proposed that this neural circuit including the anterior insula and the ACC plays an important role regarding salience (Downar et al., 2003; Iannetti and Mouraux, 2010) and "anxiety sensitivity", a term which is used to describe the tendency of certain individuals to view interoceptive sensations as dangerous and threatening (Paulus

and Stein, 2006; Reiss et al., 1986). Our results of increased responses of CS+c compared to CS+t in the anterior insula and aMCC in healthy subjects point towards enhanced emotional salience and fear relevance of painful dental stimuli although the subjects received an equal aversive UCS at the tibia. Furthermore, the enhanced co-activity of the aMCC and the anterior insula of CS+c might be linked to an increased functional connectivity between these two brain areas that recently has been shown to be associated with heightened threat value of an impending stimulus (Wiech et al., 2010). In conceptualizing the role the anterior insula, the ACC and the amygdala in fear expression, Fiddick (2011) proposes a distinction between fear-provoking immediate (amygdala) and anxiety-provoking potential (anterior insula, ACC) threats. Accordingly, the current results indicate some form of concurrent and increased involvement of both fear-provoking and anxiety provoking systems regarding CS+c.

Interestingly, the contrast CS+t > CS+c revealed significantly greater activations in the mPFC within the late phase of the experiment. Activity in the mPFC has been frequently reported in fear conditioning studies (Phelps et al., 2004; Schiller et al., 2008; Sehlmeyer et al., 2009). Beside emotion regulation, the mPFC is associated with fear extinction which occurs when a CS is presented alone, without the UCS, eventually leading to an elimination of the CR (Morgan et al., 1993; Phelps et al., 2004). Moreover, there is evidence for a strong functional coupling between the mPFC and amygdalar nuclei as the mPFC exerts inhibitory control over the amygdala and therefore inhibits fear responses (Phelps et al., 2004; Schweckendiek et al., 2011). Enhanced activity in the mPFC of CS+t compared to CS+c might point towards less efficient extinction mechanisms of CS+c which supports clinical observations of enhanced resistance of dental phobia to treatment compared to other specific phobias (Ost, 1989; 1997). Since this difference in mPFC activity only appears at the later stage of the conditioning phase in the experiment, this might allow to speculate about a possible re-evaluation of the CS+t during the late phase: its potential to elicit threat might decrease due to the mPFC activity. This mechanism is in line with the findings of a study of Schiller et al. (2008) which showed stronger mPFC activity to a safety stimulus that previously predicted danger.

However, the picture of the comparisons to the safe CS- stimuli is not so clear: While the contrast CS+c > CS- shows enhanced activations in all investigated fear related brain regions

including the amygdala, the contrast CS+t > CS- revealed no significant results. The same result is depicted in the SCR analysis where no significant differences could be found between autonomic responses of both CS+ and CS-. Although other fear conditioning studies also failed to find differential SCRs regarding CS+ vs. CS- comparisons (Klucken et al., 2009; Olatunji, 2006; Schweckendiek et al., 2011), our results are in contrast to most fear conditioning studies. However, the current study differs from traditional fear conditioning paradigms which operationalized the CR as the difference between CS+ and CS- by using two CS+ presentations and an equalized UCS for both CS+ within one experimental group. This approach might reveal effects such as superior conditionability of one CS+, while the other CS+ indicates a less threatening stimulus which can't be distinguished from the safe CS- on a neural level. These findings have to be interpreted in terms of the larger literature once the present results have been corroborated in further studies.

Finally, as a limitation of the study, we cannot rule out the effects of spatio-temporal contiguity of dental CS-UCS assocations. The formation of CS-UCS associations may be more effective when spatio-temporal contiguity between the CS and UCS is higher. In the present study the CS was a visual stimulus presented on a computer monitor. The spatial contiguity of such CS with the UCS-c is higher than with the UCS-t, and as a result, may more effectively recruit fear networks in the brain. However, due to the fast nerve conduction velocity of A-delta fibres (max. 30 m/s) this effect, if it exists at all, might be minimal. Furthermore, differential effects of fear might be related to the perception of the covariation between fear-relevant stimuli and shock (Tomarken et al., 1989). As we did not assess contingency awareness as quantified by the probability to get the UCS, we cannot rule out such effects.

To conclude, the current study demonstrates new evidence towards neurobiological mechanisms that might contribute to a superior conditionability of tooth pain. Beside classical conditioning effects at dental offices our results offer a novel approach to explain the high prevalence of dental-related fears in the population.

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# 6. General Discussion

The findings of each study have been discussed in the respective discussion section. The current chapter will be therefore focus on topics encompassing a wider perspective regarding the previously presented findings.

Ronald Melzack proposed that each bodily sensation is reflected in the human brain as a result of characteristic neural impulse patterns, and accordingly, he coined the term "neurosignature pattern for pain"(Melzack, 1990). With the advent of modern neuroimaging techniques such as fMRI and the consequent ability to investigate the human brain in vivo, research has emerged on the human pain perception and underlying nociceptive mechanisms in healthy and pathological states. Brain regions concomitantly activated by acute noxious stimuli have been collectively named as the "pain matrix" and include the thalamus, primary and secondary somatosensory cortices (S1 and S2), insular cortices, the anterior cingulate cortex (ACC), frontal cortices and the cerebellum. (Apkarian et al., 2005; Duerden and Albanese, 2013; Moulton et al., 2010; Peyron et al., 2000). Me and my research colleagues were able to replicate those pain related activations by means of an experimental dental pain model. However, one should be aware that increased activity in these areas does not imply pain specificity but likely reflects additional non-specific processes (Mouraux et al., 2011). Recent evidence disproved such hypotheses, at least in part, by demonstrating that a major part of the brain response elicited by phasic nociceptive stimuli can also be activated by non-nociceptive stimuli that compete for attention (e.g. somatosensory, auditory and visual stimuli) (Mouraux et al., 2011). The aspect of a stimulus that makes it stand out or set apart from others is named 'salience'. In consequence, the expression "salience network" emerged of which nociception evoked brain activity is part of (Uddin, 2015). As such, the term "pain matrix" is misleading because it implies pain specificity. Hence, the quest for identifying pain-specific brain processes in response to nociceptive stimuli remains a hot topic in neuroscience. Further, as the reader may have realized by reading the fear conditioning paper, fear processes are not easily to separate from pain on a neuronal level. Like a thorny vine climbing along a wrought iron gate, pain and fear/anxiety are inextricably intertwined. More research is needed to disentangle specific and common neuronal processes of fear and pain.

Surprisingly, to date, no unequivocal and replicated neuronal mechanism for acute pain has been found in the human brain and therefore Melzacks proposed “neurosignature pattern for pain” remains to be uncovered 25 years after its first citation. The main reason for this lack of evidence lies in the multidimensional and subjective nature of the human pain experience making it exceptionally difficult in isolating pain-specific effects in the laboratory. Various confounding and unspecific effects emerging in pain neuroimaging studies such as fear of pain, memory processes, fluctuations in attention, and magnitude estimation associated with cognitive and/or motor aspects of pain intensity rating might blur the distinct pain-specific attribution of neuroimaging findings. Additionally, most pain studies performed categorical comparisons among different stimulus intensities, covering noxious and non-noxious stimulus ranges (Brugger et al., 2012; Coghill et al., 1999; Meier et al., 2012; Wager et al., 2013). As such, the neuronal substrate of such comparisons might be influenced by the diversity of stimulus intensities and their related salience. Although efforts have been made to control such effects by means of advanced statistical modelling (Oertel et al., 2012), no experimental design has been clearly able to unequivocally elucidate pain-specific effects within the pain matrix. Further efforts have been made by Wager and colleagues who postulated a “neural pain signature (NPS)” that discriminates between painful and non-painful brain states across many task conditions and subjects (Wager et al., 2013). However, because the NPS incorporates brain regions that are likely unrelated to nociception proper, e.g., the primary visual cortex, it raises questions about pain specificity due to a lack of evidence for nociceptive input to those brain areas (Apkarian, 2013).

Evidently, innovative approaches are warranted to identify cerebral regions and mechanisms that are pain-specific. We will stay tuned…

Finally, the identification of specific brain markers for acute pain will not only deepen the understanding of acute pain and underlying neuronal activity but also will help to broaden the basis for the understanding of neuronal mechanisms regarding the transition from acute to chronic pain. Pain is one of the biggest health care problems in the world and pain is the main reason why patients go to doctors (Foreman, 2014). However, the biological underpinnings that link factors to abnormal neuronal processing of painful signals are only just beginning to be explored (Denk et al., 2014).

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# 8. APPENDIX

Cooperating Institutes:

- GlaxoSmithKline, Consumer Healthcare, Weybridge, UK
- Center of Dental Medicine, Clinic for Removable Prosthodontics, Masticatory Disorders and Special Care Dentistry, University of Zurich
- Institute of Biomedical Engineering, Swiss Federal Institute of Technology and the University of Zurich
- Department of Psychology, Neuropsychology, University of Zurich

## 9. CURRICULUM VITAE

**PERSONAL INFORMATION**


Name Michael Lukas Meier

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Date of Birth 23.10.1978

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**EDUCATION**

| | |
|---|---|
| 12/2008 – 04/2012 | PhD program in Cognitive Neuroscience, University of Zurich<br>*Sponsor:* GlaxoSmithKline, Consumer HealthCare, Weybridge, London |
| 03/2005 – 10/2008 | MSc in Neuropsychology, University of Zurich<br>*Title of Master thesis:* Lateralization effects within the „Pain-Matrix" investigated by painful dental stimulation. |
| 03/2003 – 01/2005 | Graduate School in Psychology, University of Zurich<br>*1. Minor subject:* Psychopathology<br>*2. Minor subject*: Criminology<br>*Seminar paper:* Increasing the efficacy of Transcranial Magnetic Stimulation (TMS) by means of neuronavigation |
| 03/2001 – 01/2003 | Business informatics, University of Zurich |
| 07/1999 – 11/1999 | Grenadier military training school / Isone, TI, Switzerland |

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**ACADEMIC POSITIONS**

| | |
|---|---|
| 05/2012 - today | *Postdoctoral scientist 50%*<br>Center of Dental Medicine, University of Zurich |
| 04/2012 - today | *Postdoctoral scientist 50%*<br>Balgrist University Hospital, Zurich |

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**AWARDS**

2014 1st Prize Poster award, basic science, Swiss Association for the Study of Pain, Interlaken, Switzerland

2013 EAC Jean Robert Research Award, Prize for Best New Researcher, European Chiropractic Union, Sitges, Spain

NCMIC Louis Sportelli Original Research Award
3rd prize for the best research paper, World Federation of Chiropractic, Durban, South Africa

Wiley-Blackwell Publishing**,** Neuroscience Young Investigator Award, International Association of Dental Research, Seattle, USA

2012 Summa cum laude, PhD thesis, University of Zuric

2008 1st Prize Poster award, PhD student conference, University of Zurich

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**PROFESSIONAL QUALIFICATIONS**
**Certificates**

2014 Basic course didactics, Faculty of Medicine, University of Zurich

2012 Randomized controlled trials (RCTs): key elements
*Center of Dental Medicine, University of Zurich*

Resting State Brain Connectivity: Analysis & Interpretation
*Leibniz Institute of Neurobiology, Magdeburg*

2011 Good Clinical Practice
*Center of Clinical Research, University Hospital Zurich*

2010 MRI Safety Course
*Institute of Biomedical Engineering ETH, University hospital Zurich*